



Hopkins & Biochemistry

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F. G. HOPKINS, 1936.

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## FOREWORD

by the President of the Congress,

A. C. CHIBNALL, F.R.S.

Sir William Dunn Professor of Biochemistry, University of Cambridge

WHEN the Committee of the Biochemical Society decided to convene the First International Congress of Biochemistry it seemed appropriate that the Congress should be held in Cambridge, on account of the close association of the University there with the work of the late Sir Frederick Gowland Hopkins. No English scientist had done more than he to further the development of biochemistry. Sixty years ago, when Hopkins began to consider the problems of living organisms, the exponents of biology and chemistry had very little in common. Those interested were biased strongly in favour of one side or the other, and Hopkins was one of the few pioneers who had the insight to realise that it should be possible to think about the organism in terms of chemical mechanisms. This led eventually to the emergence of biochemistry as a distinct discipline; and it is because the life of Hopkins illustrates so vividly the growth of this new science that the Congress Committee has thought fit to present to every member attending the Congress a copy of this book.

The book itself is a miscellany of Hopkins' own writings and appreciations of his work by others. His autobiography has a twofold interest: as an illustration of his own development and as a reflection of the difficulties confronting one who sought to enter a profession by any but the orthodox channels. Fate destined him for many monotonous years of analytical chemistry before he was permitted to undergo the normal medical training. Yet irksome as this must have been, it provided him with what was in those days almost a unique background; and in his two outstanding addresses of 1906 and 1913 we find him extolling what he calls "the art of analysis," and preaching the need to develop new techniques to help solve the problems confronting the biochemist. One cannot help feeling that had his earlier life been so cast that he could have undergone a more conventional training he would, in any case, have been an outstanding man in science or medicine, for "genius finds its own special expression in spite of circumstances"; but he would almost certainly

not have become a leading exponent of the new composite discipline called biochemistry, and the world would have been the poorer.

Many of Hopkins' earlier addresses and scientific communications have become difficult of access, and I welcome the opportunity now given to make them more readily available to the younger as well as the more senior workers in biochemistry. Of his writings even the earliest retain their freshness and carry the stamp of his intellect in its full vigour; to me they have a special value as I came to know him well only after his retirement in 1943. The appreciations of his senior colleagues given in this book show the warm affection which he inspired among those who knew him well; and the collection as a whole will enable those who never knew him to catch some glimpse of his personal charm and prophetic power.

I am confident that I am voicing the feelings of all members of the Congress in expressing gratitude to Hopkins' senior colleagues for this worthy monument to the memory of one under whose guidance they worked for so many years. It is a poignant thought that the appreciation by Dr. Marjory Stephenson was penned by her only a few weeks before her own tragic death. Her contribution is a warm, human appreciation of his leadership; but I would like to remark that her own life as a pioneer in chemical microbiology was the greatest tribute she could have paid him, for it exemplifies his power of inspiring greatness in others.



# Autobiography of Frederick Gowland Hopkins

1937 ÆT. 76



## AUTOBIOGRAPHY OF SIR FREDERICK GOWLAND HOPKINS

### *Eastbourne*

THE first ten years of my life were spent alone with my mother at Eastbourne.\* I have vivid memories left from some of those years but will recall a few only, choosing such as concern events which seem at least to have left some influence behind.

We lived in a house still known as Cavendish House, situated directly opposite to the pier. Among my memories is that of seeing the first pillar of the pier screwed into its base. A wooden structure with projecting horizontal spokes was attached to the pillar and six Coastguards, one to each spoke, walked round and screwed it home. On the top of the structure sat another Coastguard in a tarred straw hat, who vigorously played a fiddle as he rotated. The progress of the pier construction profoundly impressed me, and I cherished a hope that I might grow up to share in great enterprises of the kind. I would certainly, I thought, be an engineer. Nevertheless, I displayed less in the way of mechanical ability than most boys of my age. I have no memory of making anything with my own hands either in those days or in my later boyhood, and indeed all through my laboratory life I have felt the lack of constructive skill, admiring it so much in others. I became indeed rather bookish towards the end of those years at Eastbourne. I read then my first novel—*David Copperfield*—and revelled in the pathos and sentimentality found in it, being not in the least amused by its humorous side. I fear I have never grown out of a liking for the sentimental in literature and the drama. I was very early an adept at writing doggerel verse, of which I still possess samples. They seem precocious but display no suggestion of budding genius! Just one circumstance in these early days may have revealed and encouraged some bent towards science. My father had possessed a good compound microscope and a not very efficient astronomical telescope. My mother did not reveal to me the existence of these until I was eight to nine years old, but then I was allowed limited contact with them. With the telescope I could do little without the help that was lacking, but the microscope gave me pleasures to which the chief drawback was that they could be shared with none. Living by the shore it was easy to apply the instrument to many things of interest. I felt in my bones that the powers of the

\* F. G. Hopkins was born on 20th June, 1861. This and the following footnotes are those of the Editors.

microscope thus revealed to me were something very *important*—the most important thing I had as yet come up against; so much more significant than anything I was being taught at school. I saw vistas ahead, but unfortunately I could not discuss them. I had the kindest of mothers but she was a complete stranger to the world I was dimly visualising. Though I had the occasional company of a cousin of about my own age I was for most of those days a lonely youngster.

I went from the time I was six to a dames' school, "Grove House," which I think from the teaching point of view was better than the average. There, however, I was the only day boy, with little chance of finding a chum among the boarders, who were never allowed out save for formal walks *en crocodile*. Unfortunately, towards the end of my stay there I realised for the first time the existence of cruelty in the world. I learnt, secretly and gradually, of appalling bullying in the dormitories which, as a day boy, I personally escaped. Of the two eldest boys in the school, one, though I suppose he could not have been more than 13 or 14 years of age, was a sadist of unbelievable ingenuity in method, and the other truckled to him. Some of the youngsters were utterly broken in health and spirit by continued ill-treatment. The maiden sisters who kept the school were long blind to all this, but it was somehow revealed to them shortly after I left. My only justification for recalling this circumstance is the profound effect it had upon myself. Till I realised what was happening at the school I had met nothing but kindness in the world, and the discovery of cruelty filled me with horror. I became suspicious of my kind and even more self-centred than loneliness had previously made me. The effects lasted long, certainly throughout the rest of my boyhood. Had I myself been sent to a boarding school to mix with all kinds of boys I should doubtless have learnt to view bullying with a better sense of proportion.

### *Enfield*

In 1871 my mother and I left Eastbourne to live with a remnant of her own family—my maternal grandmother and an unmarried uncle.\* Shortly after we moved to the suburb of Enfield where, after a year or two, my uncle built a house on the "Ridgeway," which then was in countrified surroundings, and which became my home for twenty years.

My mother shrank from sending me to any sort of boarding school and, with morbid memories of the Eastbourne revelation, I hated the

\* James Gowland.

prospect myself. After discussions, which I was vaguely aware were in progress, and partly, as I was afterwards told, because of the great reputation of Abbott, then the headmaster, the City of London School was chosen for me. I went there at the age of  $10\frac{1}{2}$  and left, in untoward circumstances (as will be seen), at 14. The school building was then of course still on its original site in Honey Lane, so closely surrounded by other buildings that scarce a ray of sun could enter any of its windows. My history there during the first three years was uneventful. I started in the lowest form, then the "4th Junior," and, without missing a remove, arrived in the "3rd Senior" at the beginning of my fourth year. This was a performance neither deserving nor receiving special notice. It is, however, perhaps worth mentioning that during my third year, in the pass list of the examination in chemistry (a school and not a form examination), the First Class contained three names only, those of the two sons of W. H. (afterwards Sir William) Perkin and my own. I remember being startled at this result, for though I had attended all the lectures on chemistry (delivered by Henry Durham—an exceptionally good teacher), I had not specially prepared myself in any way for the examination, not even possessing a chemical textbook. I suppose it is legitimate to assume that this showed some special aptitude for chemistry, though at the time I was certainly unaware of it myself. In any case, it was not to be further displayed at the City of London School. In what I think was the following term I was awarded, in the "3rd Senior," the class prize for English and a prize for the best "Essay" (really a dramatisation of Scott's *Old Mortality* in blank verse).

I was not fated however to receive my prizes, in due form and on the proper occasion, from the hands of Dr. Abbott, otherwise I might then have won his notice. Before the end of the term, when the right occasion arose, I had wholly absented myself from the school for several weeks. I had become a truant of the completest kind.

Trying to view myself as I was then from the standpoint of to-day I find it difficult to understand my youthful conduct. In essentials I believe I was a guileless youngster and I certainly got up to no mischief during the weeks of my truancy, which soon became wearisome enough. Country walks, visits to museums, one or two to the docks (with no thoughts of taking ship!) and, though I have since been told that I could not have been admitted when so young, visits to a public library which I feel sure was that of the Guildhall, though so dangerously near to the school: in such ways the days were passed. The essential sin, though it involved no verbal lie, for no

questions were asked, was of course the daily pretence at home. A defect of character never wholly overcome was doubtless displayed in my inability to face the ordeal of the confession which I daily longed to make. Ever since, more than most I think, have I suffered from temptation to postpone the unpleasant. Revelation of my truancy only came, and then necessarily came, at the beginning of the following term. An intervening summer holiday spent with my mother and uncle in Wales was one of misery for me; I knew what was ahead. There came a painful interview between Dr. Abbott and my poor mother, to share in which I was ultimately called. I was informed that I was not to be expelled but that my removal was advised. These words were the only ones ever addressed to me either by Abbott himself or any other master.

If by digging deep in memory I endeavour to recall what was the state of mind which first led to this lapse from right conduct, so soon regretted, I come honestly to the conclusion that it was sheer boredom with my life at school. Though doubtless the blame was chiefly due to me I feel that circumstances should share it. I made no friends, partly because my suburban journey to and fro from Enfield meant that I arrived at the school only just before the beginning of class and, on parental instructions, always caught the earliest possible train when school was over. There was each day, it is true, half an hour's interval for lunch, when all the junior classes mingled; but this meant a crowded, riotous time in an underground corridor where the tuck shop was to be found. The interval gave little opportunity for the discovery of kindred souls. My brotherless life at Eastbourne and memories of that discovery at the dames' school left me still rather shy of my kind.

It is, moreover, literally true that throughout my days at the school I received no single personal word from any master. There was no suggestion that anyone took the least interest in my progress. Indeed, I think it quite fair to say that at that time (the earlier seventies), while very high standards had been established by Abbott in the upper forms, those of the lower school were much inferior. The classes were far too large for single form-masters to teach adequately. The masters that I knew were all old and near their age for retirement. J. S. Macdougall, C. N. Woodroffe, Thomas Sharp and Joseph Harris were my instructors and all had been teaching for more than 30 years on salaries which (as I have recently learnt) were discouragingly small. I think they had lost their enthusiasms. All retired not long after I left. I think further that the general management of the lower school

was slack just then, for although during my truancy I was absent for some weeks no enquiries concerning me reached my home.

I have perhaps made too much of the incident (trivial in itself) of my fall from grace at this time; but clearly it greatly affected my subsequent fate. One more form remove and I should have come into a very different atmosphere. Inspiration from Abbott himself together with that from Rushbrooke and Cuthbertson must surely have greatly stimulated me as it stimulated many others. Perhaps my career might then have followed quite different lines.

Moreover, the incident of my truancy unhappily left my people—for a short time at least—with the fear that I had more than my share of original sin and therefore might require exceptional treatment. This perhaps led them to choose the private school to which I was next sent. Of this I need say little more than that from an educational point of view it was not a happy choice. The proprietor was a worthy man without a university degree and without anything which a degree should stand for. His staff was small and inadequate. The teaching was wholly uninspired. Of the classics we heard little or nothing, and our mathematics were elementary. The science teaching amounted only to an occasional reading by the Head of a chapter of Huxley's *Elementary Physiology*, with comments by himself in the style of Paley. There was a chemical laboratory, but in my days nothing was done there. It was the custom at that time for private schools of the kind to send pupils annually for examination by the "College of Preceptors." When I went up I somehow managed to secure one of the prizes for science as awarded by that august examining body. This again surprised me and no less my schoolmaster. He promptly mentioned the success in his advertisements however. After three years at this seat of learning my school days finished. I was happy enough there, and made a few desirable friends among my contemporaries, but of education in any adequate sense of the word I received very little.

### *Insurance Interlude*

At seventeen a career was to be chosen for me. My uncle, my guardian *de facto*, had mainly City connexions and it was in that environment that a possible future was pictured. An old friend of the family had influence in the insurance world, and with his help I found myself in my seventeenth year on a stool in the London office of a provincial insurance company. I occupied that stool for six months only. I don't know whether it was because of my failure or my success in the office that the friend just mentioned was led to

advise that I should be given a chance (so he put it) to make more immediate use of my brains! I hold him in grateful memory, for somehow he acquired and expressed to me and to my people the opinion that I was meant for a scientific career.

### *An Analyst's Laboratory*

So encouraged, I myself elected for science, and my father's cousin—his one-time chief chum—Fritz Abel (afterwards Sir Frederick), who had till then doubtless forgotten my existence, was approached for advice. He at once said "Cambridge!" One day just then, however, my uncle met a City acquaintance who was connected with the firm of Allen and Hanbury, and so was supposed by the family to have the right sort of knowledge for guidance. He expressed the opinion that "only theory was taught at the University," remarking at the same time that he had an acquaintance in practice as a consulting chemist with a vacancy for an articled pupil. He persuaded my uncle that only in such a laboratory could the practical knowledge be obtained which was necessary for one who had his living to make.

There followed for me three years in the rough and tumble of a very busy analytical practice. These years taught me something perhaps which might not have been learnt elsewhere, for instance, how to obtain results in the shortest possible time; but intellectually they were nearly sterile years. An able assistant taught us methods empirically as occasion arose, but without system and always only for the immediate calls of the practice. In fact, the "articled pupils," who paid quite respectable premiums for their privileges, became in a month or two merely unpaid assistants who worked from 9 a.m. to 6 p.m. As I myself had the rather long journey to and fro from Enfield each day there was little leisure for self-education in basal chemical knowledge. Our chief had little touch with us. He often passed through the laboratory, but in silence, to reach a certain wash-hand basin, and his ablutions were strangely frequent. On a shelf above the basin there stood always a reagent bottle with a label which did not betray its contents. Our employer, indeed, was prone to consume whisky in his working hours. He was doubtless very clever in his own way, but not the nicest or the most honourable of men. The otherwise wholly commercial atmosphere of the place was somewhat mitigated for me by a friendship with one of my fellow pupils, who had real intellectual interests and helped to awaken mine. He ultimately left chemistry for art, and reached some distinction as a painter. This was Herbert Draper.

I have written plainly of this place as there are very few now left alive who could identify it. The years there passed were important years in one's life, but save perhaps for some manipulative skill, I gained little from them. At their close the question arose, "what next?" But now I myself took charge of my future, helped by an inheritance—no large one—from my paternal grandfather, who long outlived his son, but of whom I at no time saw more than very little.

### *South Kensington*

I was now\* acutely aware that I wholly lacked any proper scientific education, and looking round, decided upon what was then, for one in my position, the easiest course; I went to the Royal School of Mines at South Kensington. It was my special hope to profit from the teaching of the elder Frankland, then at the zenith of his reputation. I felt it was too late to start the complete courses for the diploma of A.R.S.M. and decided, perhaps unfortunately, to join the chemical class only. Alas, I saw little of Frankland. Some readjustment in the chemical department was taking place just then and he did little teaching during the term. He started, I recollect, to come and talk to members of the practical class one day. He had got as far as my next neighbour when a messenger fetched him away, and, except in lecture, I never saw him again. Moreover, after he had delivered two—or at most three—lectures that term his course for some reason stopped. Yet those lectures were the only educational lectures on chemistry to which I have ever listened!

I did well in the end of term examination and, whether for this reason or another, Dr. Percy Faraday Frankland, then a young man some three years older than myself, invited me to become his assistant at a private laboratory which he was just then starting. I felt that the invitation was a great honour and accepted it at once. The laboratory was in a private house in Pembridge Square. It comprised two small rooms which, some years before, had been used as a laboratory by Dr. Gladstone. The work consisted mainly of analysis of steel rails and creosote samples for one of the bigger railways. I worked alone and for rather long hours. The dark winter days were rather grim. This appointment, however, proved to be but another interlude in a career with a still uncertain future. At the end of about six months some events brought the railway work to an end; Dr. Frankland—about to be married—made other plans, and I was out of a job.

\* At 21.

I then realised that it would be well for me to obtain some recognised professional qualification and decided to aim at the Fellowship of the Institute of Chemistry, a body which was then (1882-83) growing in influence.

I went therefore to University College, and thinking it likely to be more useful for my purpose than any pure science course, joined the technical class then taught by Charles Graham, a somewhat dour Scot. His lectures were of little use to me, and were then but little attended. He gave a course, I remember, on the chemistry of bread-making, at which, on more than one occasion, I was his sole audience and I was not faithful. The practical class, however, followed by only five or six students, although it covered ground most of which was familiar to me (food analysis, etc.), was useful in preparation for the practical examination, success in which (fortunately for me) was then all that was required for admission to the Associateship of the Institute. The examination when it came proved to be a turning point in my life. Till then I think it is fair to say that I had enjoyed little enough in the way of good fortune in my attempts at a career; ever since I have had even more than a fair share of it.

### *First Years at Guy's*

I did exceptionally well at the examination and Dr. (afterwards Sir Thomas) Stevenson, the Medical Jurist at Guy's Hospital and Home Office expert, came somehow to hear of my performance, probably through his own connection with the Institute of Chemistry. He invited me to become an assistant in his laboratory. I accepted with delight and then began five years of work there which were happy, interesting years for me. They also gave me—as I shall relate—opportunities for preparing for the academic career which, though somewhat late, I was ultimately to enjoy.

The laboratory was a single room, being part of the structure of the old so-called "clinical ward" (now pulled down; "clinical" *par excellence* because of some special privileges it possessed). There was a second assistant, senior to myself, Richard Bodmer. He had no scientific interests outside his immediate job; but he was a very fine musician, and from him I learnt something of music and a good deal about the musical world of that day. When I joined the laboratory the famous Lamson case was just finished, and aconite remained a subject of interest; when I left it—or immediately after—the equally notorious Maybrick case began. In between I carried out the analyses in a large number of cases involving the detection of a considerable

variety of poisons and often the disproof of the presence of any such. I might describe many cases, but for special reasons I best remember two. One was the Bartlett case, in which a wife was accused of killing her husband by pouring liquid chloroform down his throat. I estimated the chloroform in the stomach contents and there was plenty of it. I chiefly remember the case however because it called forth from Sir Edward Clarke, who defended the woman, what I think must have been the most wonderful example of forensic eloquence ever heard in court. Stevenson gave expert evidence for the prosecution and easily withstood Clarke's cross-examination. There was, however, some uncertainty as to how the chloroform was actually administered, and, in any case, if the prisoner were guilty a clerical paramour should certainly have been in the dock with her. Of the latter circumstance Clarke made much, as of other points of weakness in the Crown case. It was not, however, his technical skill that was so striking, but the overwhelming eloquence of the speech as a whole. I was in court throughout and recall it vividly, though it was delivered more than fifty years ago. When Clarke sat down there were deep murmurs of satisfaction, and when very shortly afterwards the mesmerised jury brought in a verdict of not guilty the folk in court got entirely out of hand and cheered vigorously; many who had hats available threw them into the air. A big waiting crowd outside the court echoed the cheers, for the public were indignant that the woman had been placed in the dock alone.

The other case I will recall had, like the Bartlett case, no particular toxicological interest or difficulties, but there was some drama in its outcome and I myself was more particularly concerned with it. A Polish Jew in the East End of London, Lipski by name, whose occupation involved cleaning and polishing the silver mounts of umbrellas and walking-sticks, was accused of murdering a woman whom he found asleep in bed, by pouring nitric acid down her throat. There was no doubt about the cause of death. The woman's mouth, gullet and stomach were dreadfully eroded, and it was, of course, easy to prove the presence of nitric acid in the tissues, as also in stains on the bed clothes and on the boards of the floor. Lipski was found guilty, but the evidence against him was circumstantial. He was known to use nitric acid in his trade and to have had some trouble with the woman, but (if I remember aright) the proof that he, and no other, could have done the deed was not quite conclusive. At least, the public thought so, and when Lipski was condemned there was a press campaign in his favour, and Mr. Matthews, the then Home

Secretary, was bombarded with petitions for his reprieve. Matthews remained firm, however, strengthened by evidence that was provided by myself after the trial. In the laboratory there were still portions of the stained bed clothes, and of Lipski's coat, also stained with acid. Stevenson had testified to the presence of nitric acid in those stains. On looking at them one day, however, it seemed that after all they were not quite typical of stains due to nitric acid alone, which on most fabrics are yellow in colour. These were somewhat more red. Sure enough, on testing, I found that appreciable quantities of sulphuric acid could also be obtained from them. The same was true of the stains on the floor near the woman's bed. Now nitric acid is (or was) occasionally, though by no means commonly, adulterated with sulphuric acid. The police were informed of the new fact, but were by then I think unable to obtain from Lipski's room a sample of the acid used by him in his work. They made, however, a number of purchases of nitric acid from different shops in the neighbourhood. I tested the eight or ten samples brought by them to the laboratory and only one of them contained sulphuric acid. This was sold by the shop whence Lipski always obtained his supply, and he had purchased acid there just before the murder! Lipski ultimately confessed his guilt. Many of the cases that went through Stevenson's hands in those days were interesting because of the toxicological problems they presented and I attained to skill in the isolation and identification of alkaloids. There was work of other kinds to do in the intervals between cases, as Stevenson was public analyst for more than one local authority.

My chief friend during those years and for some time after was John Wade.\* He then held the minor post of chemical lecture assistant, but he was a man of marked ability and sterling character and before I left Guy's for Cambridge he had himself become lecturer and written an exceptionally good textbook of organic chemistry. His folk were not well-to-do but he had a cultivated home and his father

\* Another friend of this early period was Charles (later Sir Charles) Martin, who has recalled the following facts: "I first met Hopkins at Guy's in 1883 . . . he was then a highly skilled chemist; I was a first-year medical student. Finding I was interested in chemistry, he kindly allowed me to potter about in his laboratory at odd times, and when he was not too pre-occupied he would discuss chemical problems in physiology which interested and perplexed me. I remember the consideration he gave to my crude imaginings, and the delicacy with which he demolished them. The benefit of these unceremonious talks was gratefully appreciated, and I became perhaps the first of his loving pupils. As we became intimate in those old days at Guy's, I had the youthful effrontery to urge him to abandon his career as an analyst, study medicine, and subsequently devote his talents to researches in physiological chemistry. At first he was amused, and then attracted to the idea, but . . . for financial reasons the proposition was, for the time being, impracticable." (*Lancet*, 1947, (1), 730).

and sister were gifted musicians, the former being also an excellent amateur painter. I greatly profited from visits to their house.

Wade and I both greatly regretted that we lacked a degree, and, one day, some time I suppose during my second year with Stevenson, we together rather suddenly decided to become external students of the London University and try that unguided path to Parnassus. I don't quite remember in whose mind the determination first arose. It was probably in Wade's.

The task ahead was stiff. Our working hours were long; holidays, in my case at least, were short, and we both realised that family life made considerable demands on our evenings at home. Doubtless very many other external students have attained to London degrees in circumstances no more favourable than ours. We at any rate reached our aim without a set-back, and in minimal time.\* At one stage only had we to seek some external help. We could not provide ourselves with the necessary materials for practical work in biology, so we joined the evening class provided by Birkbeck College and were grateful to that Institution for the help it gave us. For us both, however, the first fence on our road was the stiffest. Wade indeed had had a pretty sound school education; he went to St. Olave's after Rushbrooke had left the City of London School to become Head of the former. He was also a year or two nearer his school days. In my case six years, with small leisure, had elapsed from the time I left the private school, to the qualities of which I made earlier reference. It was not easy in these circumstances to "get up" all the subjects of the kind required for the London Matriculation. I can fairly say that during those years, and, indeed, so far as reading was concerned even during my later years as a medical student, my chief place of study was a compartment in a suburban train (there and back,  $1\frac{1}{2}$  hours each day!). I used every minute of those journeys, learning how to concentrate, and acquiring skill in avoiding travelling companions who might interrupt. Thus it was that an academic career became a possibility. Like so many others, I owe this to the external student system of London University; and must remain deeply grateful for the opportunity which it gave me. I am bound to say, however, that obtaining a degree on the lines I had to follow involved some little mental and moral damage. Long continued reading (if not cramming) *ad hoc* at my then age (24-27) without reference to one's own tastes or to the development of such innate special abilities as one might have, could not fail to do some permanent harm to the

\* 1887.

mind. I think I was born with a memory easily stored for a time (therefore making examinations always easy for me) but not tenacious. The years just mentioned tended to increase this defect, and I must confess that even during later years I have too often had to read even my own subject for *ad hoc* purposes (lectures, etc.) rather than for sound mental storage. This and the lack of early education in the basal aspects of science has made me, I feel, an amateur intellectually.

Having some capital at my disposal I now (1888) decided to join the Medical School at Guy's. In this I was encouraged by my employer (by then Sir Thomas). He suggested that I might possibly qualify to succeed him in his position at Guy's. I might remark here that after his death when I was already at Cambridge I did (at the wish of the Stevenson family) accept the appointment of Home Office expert. I became for a while junior in that office to Dr. Willcox (now Sir William) and dealt personally with a few cases. I had lost taste for the work, however, and my laboratory was ill-equipped for it. I was indeed much relieved when some adjustments in the policy of the Office brought my appointment to an end.

To the kindness and good opinion of Stevenson I owe much indeed. I was a frequent visitor to his home and found real friendship there, and in working hours he was the most considerate of employers. He knew the subject of forensic medicine from A to Z and was a most admirable witness in court. On the other hand, his knowledge of chemistry was limited, and he was not a good performer in the laboratory, at any rate not by the time I came to know him. Bodmer and I were indeed always rather anxious when it fell to him to confirm some final test in an important case. Untoward happenings sometimes occurred. He realised this, I think, and seldom interfered in our analytical manipulations. I would like, however, to pay a tribute to qualities in Thomas Stevenson which were really great.

Some aspects of my career as a medical student were exceptional, apart from the fact that it started when my age was nearly 28. It began with an unusual circumstance. Directly I joined I was awarded the research studentship founded in memory of Sir William Gull. Of this I was the first tenant, and I held it, I think, for three years. It was among the first—if not the first—of such research studentships in the medical sphere. I suspect that the reason for its falling to me must have been some very strong backing from Stevenson, for I was unknown or nearly so to the rest of the staff. I had much reason for gratitude to the electors to this studentship. Its tenure gave me something of a cachet from the first, together with privileges of value.

It was, however, a tough job to do it justice during the business of getting qualified.

My first task was to tackle the Intermediate M.B. London, and, for the first time in my life, I listened to educational lecture courses from qualified teachers. Up till then, though not much longer, the courses in physiology and anatomy at Guy's were delivered by members of the clinical staff. The rapid growth of the former subject made it difficult for any one of them to lecture upon it with adequacy. In my year the course was given by the surgeon, Golding-Bird, and I am bound to say that his lectures were of a perfunctory sort. This was hardly to be wondered at if the tradition was true that when he met in the ward a fresh set of dressers he would say to them: "You fellows are now going to face the real thing. You can forget all the stuff I talked about downstairs." Some inspiring teaching in physiology just then would have made a good deal of difference to my outlook. Wooldridge, though already on the clinical staff, must, I think, have held the post of demonstrator (or perhaps assistant lecturer) in physiology that year, for he appeared occasionally in the practical class, which mainly dealt with histology, but was taken through a few chemical exercises. I remember with pleasure a pat on the back I got from him when I displayed test-tube preparations of the classical haemoglobin derivatives which were (not unnaturally) somewhat superior to those made by the rest of the class. I heard him deliver one lecture only—on his own subject of blood coagulation. He died during the following year. Starling, unfortunately for me, joined the staff too late for me to profit by his teaching but, in later years, I gained much from his friendship. He was a younger man than myself, having entered the school while I was still with Stevenson. Descriptive anatomy was then very thoroughly taught at Guy's and material was abundant in the dissecting room. I spent, perforce, a good many hours there, but, alas, I could acquire no taste for the subject. As a matter of fact, I was none too regular in my attendance on any of the pre-clinical lectures and classes. I was trying to do other things and especially to justify my research studentship. The Intermediate M.B. came easy to me however. I got the gold medal in chemistry and honours in materia medica. It is literally true that in the case of the latter I knew nothing of the subject two days before the examination. I managed in that time to cram up Hale White's textbook. The facility for passing examinations apparently lasted later in life with me than with some others. Though convenient, it was intellectually, as I have already hinted, no desirable gift. I

forgot all my knowledge of *materia medica* in a very short time and had to get up some of the subject again when the final M.B. was due. More serious was the circumstance (as will become clear) that by the time I went to Cambridge I had entirely forgotten what knowledge I had ever acquired of the details of anatomy.

Going now into the wards I became in succession dresser to Charters Symonds and Davies Colley, and ward clerk to Newton Pitt and Hale White. Except perhaps in the case of the senior surgeon, who, I fancy, tended to believe that my laboratory entanglements would lead me to neglect my ward duties, I received much encouragement and subsequent friendship from my clinical teachers.

### *Medical Work*

These were days of rapid changes at Guy's. The mortality among members of the medical staff during the last decade of the eighties was truly remarkable. Helton Fagge died in 1883, just after I went to Stevenson; Mahomed in 1884; Moxon and Carrington in 1887 and Wooldridge in 1890. There had, therefore, been rapid gain of seniority for some, while others when I joined were fresh to the wards. A happening of importance was the importation for the staff of E. C. Perry, of the London Hospital (Sir Cooper Perry), whose great energy and will-power as well as his high abilities made him a powerful force at Guy's for many years afterwards. To me he was an exceptionally good friend right up to the time I left the hospital.

I saw a good deal unofficially of Washbourne during these earlier days as well as later. While junior physician he was then introducing bacteriology for the first time into the school and I helped him occasionally in the laboratory, learning the Koch technique with him. He had no special training in the subject, but being very able, he soon mastered its essentials. He was a man of fine character and I still feel that his friendship was among the best gifts in my life. We went together (I think in 1890) to spend Christmas with the Hale Whites at Arosa. This was in the very early days of winter sports, and Washbourne was one of the very few who dared to perform on skis. Later, we went together to the Pasteur Institute for a short time, where we had a glimpse of Pasteur himself and got to know Roux and Bordet. The—then new—serum treatment for diphtheria was receiving a very thorough trial in Paris. Special wards were full of patients undergoing treatment, but never shall I forget the extraordinarily bad nursing arrangements we saw there.

Washbourne's early death was tragic, for he was a man of great promise. The untimely death of his young wife seemed to break his spirit entirely. He served in the Boer War and shortly after its end died from acute military tuberculosis.

Among my contemporaries (contemporary in standing in the school; not in age, I was four to five years older than most of them) I made a few friends of value. Among them, in particular, was Percy Allan, afterwards a very successful general practitioner in Croydon. He was a well-read young man, able, and with many real intellectual interests. He had a strong sense of humour and was a confirmed anti-sentimentalist. I was very keen on Henry James just then, and I remember trying to make him appreciate *The American* or the *Portrait of a Lady*. But he would hold his nose and say "read *Tom Jones* again my boy, *that* will do you good." In a serious sense, however, his influence was for me beneficial. He shook up one's mind when, except in limited directions, it was in danger of becoming lazy. In later years I saw him but little. He died relatively young; I think soon after the South African War.

I managed to do a fair amount of research, of a kind, both before and after I first went into the wards. I perhaps did rather more when later I was put on the school staff, but I always had a good many other jobs to do. During the former period much of what I did never reached publication. I was rather keen to carry out investigations related with problems from the wards, thinking this would be the best justification for my tenure of the Gull studentship. It will be easily understood by those familiar with such circumstances that these very often petered out without coming to conclusions definite enough, or interesting enough, to justify publication. I will give one instance out of many of what I mean. Just then, without much reason, uric acid in excess was under suspicion of being the cause of a variety of troubles. Newton Pitt had the idea that it might play a part in the causation of migraine. I estimated its excretion on a number of cases put upon known dietaries, but found no significant departures from the normal. This investigation gave me, however, the opportunity of making my first publication as Gull student. The existing methods for estimating uric acid were either inaccurate or consumed much time. I myself worked out a method which became the standard method until, much later, colorimetric estimations were introduced. I was proud of this at the time, for I perfected this method during a week's concentration on the problem.

In 1894 I obtained the Conjoint Board Qualification and the London

M.B. in the same year. Immediately after I qualified (and indeed I believe somewhat before) I was put upon the school staff. With John Fawcett I acted as demonstrator in practical physiology in the early days of Starling's appointment as lecturer. I did, in addition, a good many other odd jobs of teaching. In two or three successive years, for instance, I took practical classes in toxicology; a sort of refresher course in physiology, and at one time, in circumstances the nature of which I cannot recall, I gave lectures for 1st M.B. candidates in elementary physics and chemistry. At the same time I did my best to keep some sort of research going, and, now and then, obtained help from a pupil. In 1895-96 I was fortunate enough to do some conjoint work with A. E. Garrod, and so started a long and valuable friendship.

My clinical knowledge would have remained small indeed (as I never held a house appointment), but for having been one of the so-called "clinical assistants" (usually shortened to "clinicals"); appointments peculiar to Guy's, and most valuable. I don't know whether they still exist in their original form. They were held for six months by a group of six students, with beds in the wards of which I have spoken as being in the same building as Stevenson's laboratory. A representative of the group, changed each week, was allowed first choice of all medical cases admitted to the hospital, so that the clinicals were highly privileged. We could choose a wide variety of cases or concentrate on cases of some particular disease, according to our current desires. We had continuous and intimate contact with our own patients and some degree of responsibility for treatment, but during the six months we received guidance and criticism from four different members of the clinical staff, each taking the "clinical wards" for six weeks. The privileges of the appointment are well known to many old Guy's men. At that time at least there was nothing quite like it at other schools. I mention my tenure of it because it was of immense value to me. When appointed I made up my mind to drop everything else that could be left; read medicine hard and managed to learn a good deal.

During my last two years at Guy's I took on a task which, as it was a somewhat exacting one, needs mention. The *Guy's Gazette* had for a long time run what it called a "pathologists' column." Guy's practitioners were invited to send pathological specimens for examination, and reports upon them, made by certain members of the staff, appeared in the column. Increasing use was being made of this and its popularity led certain folk, especially perhaps Wells, an able and

enterprising chief clerk in the office of the hospital and school, to believe that the evidently widespread desire for help of this kind might be exploited. The first idea was to obtain control of an established journal which could make a speciality of such reports. One such was thought suitable, but negotiations for its acquisition were not successful. I came into the enterprise on the following lines. I was just then editing the *Gazette* for a time (I had been part editor in my first year) and so was present at early discussions. I believe I was the first to suggest to Wells that success would come not from reporting through a journal but by direct reports sent through the post. Upon such an enterprise it was determined to embark, those specially concerned being two members of the clinical staff and Wells. Now a few months before this John Wade and I had together hired a room in Denman Street, S.E., near to Guy's, and had fitted it up inexpensively as a research laboratory for ourselves. We vacated it for the purposes of the new enterprise, and in it the "Clinical Research Association" first had its home. Just before that time I had indulged in the luxury of a paid research assistant (I fear I was expending capital pretty fast!), an excellent fellow called Coram. Him, too—for the sake of his future—I transferred to the "C.R.A.," and he was with them for many years afterwards. The first secretary I also provided; a lady from Enfield whose family were very old friends of ours—a Miss Kendall. The enterprise was a great success from the first. How its existence was advertised to the medical profession I don't remember, but within a few days from the start specimens crowded into the laboratory. The anatomical reports were made by Targett, who was then surgical registrar at the hospital; the bacteriological by Pakes, while I attended to the chemical side, together with many oddments. I was paid a decent salary, but the work was hard, and usually done after 5 p.m. This meant that for a long period I could seldom get home in time for dinner, and evening leisure was unknown. The enterprise through the years of its existence must have been exceedingly profitable to those who were its original proprietors. One of the three is still alive. To say the truth, I felt a little hardship at the time. Practically no capital was required at the start, but we who organised the laboratory work were under the impression that when fresh capital was wanted we were to become shareholders, to some extent at least. When this point ultimately came up, however, such claims as we had were not recognised. I kept this work up, together with many other jobs in the medical school until, in September, 1898, I went to Cambridge.

Under Starling's rule the physiological laboratory had by this time been enlarged and an excellent chemical room provided. In this I now carried on such research as my leisure permitted, mostly side by side with J. B. Leathes, who was beginning his work on mucins and mucoids. Bayliss used frequently to come to the schools to carry out experimental work with Starling, and I often watched them so engaged. My life at the end of my Guy's days was indeed full of interest, but one's energies were scattered.

In 1893 I had the happiness of meeting her who five years afterwards was to be my wife.\* I quickly realised how great was my good fortune, for apart from that increase in life's values which sentimental attachment induces, I found in her qualities in which I was (and am) sadly lacking; the courage that minimises life's difficulties, and high efficiency in dealing with all its practical problems. She was an orphan, and our long engagement was a trial for her. Her residence with relations in Carlisle kept us apart for a time, but she determined to find satisfying activities and joined the nursing staff of the Royal Free Hospital as a so-called lady probationer. The experience she thus gained was a great help in the rearing of our children, and enabled her to render valuable services as Sister of a V.A.D. Hospital throughout the years of World War I. We were married only four or five months before the move to Cambridge, living meanwhile in a flat at the top of a high building in Lincoln's Inn Fields. On the floor below lived Ramsay Macdonald and his wife, a remarkable woman. At that time I was expecting to follow some sort of a clinical career. It is not impossible that I should have been put on the clinical staff at Guy's. I had at least the feeling that Sir Cooper Perry, whose influence was great, had that possibility in mind.

One day, however, the Physiological Society met at Cambridge and dined in Christ's College.† As, after dinner, I was emerging from the great gate, Michael Foster caught me up, took my arm, and proposed then and there that I should come to Cambridge and develop there teaching and research in the chemical side of physiology. After some agony of indecision, mitigated by the assured attitude of my wife, I took the irrevocable step and entered that atmosphere to which I had been so complete a stranger. Only a couple of months intervened between Foster's invitation and the beginning of my new job.

\* Jessie Stevens, daughter of Edward Stevens, of St. Lawrence, Kent.

† 1898.





F. G. HOPKINS AND JESSIE HOPKINS AT BREAKFAST WITH S. W. COLE. Baitsbite, 1902.

*Cambridge*

It will be recalled by any who have troubled to read what has gone before that I went to Cambridge without any training as a specialised biochemist. I had never paid the then orthodox visit to a German laboratory and, indeed, had had no contact with any master of the subject. Although such research as I was able to do at Guy's fell naturally into chemical lines, I had not expected the fate that now befell me and had not made any special effort to become familiar with the (then relatively small) current literature of the subject. It was, therefore, with some trepidation that I started lectures and practical classes for students taking physiology in Part II of the Tripos. I had to prepare for both at very short notice. I look upon it as one of my greatest gifts from fortune that somehow my teaching was appreciated from the first, and the unmistakable signs that this was the case comforted me greatly.

My debt to Cambridge to-day is very great, and especially, as I shall point out, to Trinity College, but I will confess to some disillusion when first I arrived there. The teaching of the chemical physiology had been somewhat perfunctory, especially for two or three years before my time, and laboratory equipment for advanced teaching was completely lacking, little more than the usual rack of reagent bottles and some test-tubes being available. It was not easy to make a change, and I had for a long time to adjust the class work to these deficiencies.

In particular, after so scattered a life, I had looked forward to opportunities which would at last enable me to concentrate upon one subject and learn to feel for it some sense of mastery. But this was not yet to be. Foster had warned me that I must expect to receive no more than £200 a year from his department, but explained that Emmanuel College would welcome me as supervisor of its medical students, and thus substantially add to this small income. When I made contact with the college authorities I found to my dismay that this meant teaching anatomy as well as physiology. I should have had the courage to confess to my lack of qualifications for dealing with the former. But I knew nothing of college customs, was uncertain of my ground, and I knew that I must earn more money than, apparently, the department of physiology could afford.

Wrongly, perhaps, I undertook the task and, in consequence, term time in my first year at Cambridge became rather grim. Of necessity, since I had had no opportunity of preparing them before I came, I lived from hand to mouth that year with my Part II lectures, while every hour of supervision in anatomy took me several hours' preparation,

so completely had the minutiae of the descriptive subject (which are what the medical student wants) slipped from my memory. Moreover, I had to deal for Tripos candidates also with a certain amount of comparative anatomy and embryology, concerning which my own knowledge was scanty indeed. I continued this supervision work at Emmanuel (towards the end in conjunction with tutorial work there) for 12 years. Even from the first medical and science students at the College were numerous, and during terms I took classes five days a week from 5.0 to 7.0 p.m. each day. Research during term could only be done in hours snatched with difficulty and, unlike the university teaching, college work, of course, went on in the long vacation term. I know well that many others in Cambridge were later in like case, especially the younger men when appointed to university posts without a college fellowship. That tradition in the two older universities which made college fellowships and college duties the chief sources of individual incomes—the small emoluments attached to university teaching posts, except professorships, being merely accessory—has always made, and to some extent still makes, the provision of adequate pay for imported teachers a difficult problem. Let me say here that though it was difficult during these earlier years to put all the energy I would have liked to put into the development of biochemistry in the University, I enjoyed great advantages from the close association I had with the students one taught for Part II of the Tripos: this contact with the minds of eager young men and women of exceptional ability was a great privilege and an abiding pleasure during those years.

In 1902 I was promoted to a University Readership, towards the salary of which Emmanuel College made a generous contribution, and in 1906 that College gave me the position of Science Tutor then vacated by A. C. Seward on his election to the Chair of Botany.\* Thenceforward I enjoyed an income adequate for the needs of my family. The Tutorship, of course, also involved a Fellowship. For this I was deeply grateful, for I had been eight years in Cambridge without that full association with a college, and this for one already senior in years was a circumstance involving some discomfort. I found real interest in the duties of tutor and acquired much affection for the (always responsive) young men that came under my care. The responsibilities, however, made a heavy addition to those already on my shoulders. I had a large side, and, apart from tutorial contacts, I continued to supervise a large number of my pupils (though no longer in anatomy!). Meanwhile, advanced teaching in the university

\* Hopkins had been elected into the Fellowship of the Royal Society in 1905.

classes was growing in its demands, for biochemistry was beginning that racing progress which has since continued and accelerated. I was also trying hard to keep research going.

I held the Emmanuel tutorship till the beginning of the Summer Term of 1910, when suddenly I fell ill. Whether the nervous breakdown (so-called) which then began was due solely to overwork, or whether it was largely traumatic in origin I do not know. Certain it is that uncanny cardiac symptoms began within a few hours of my striking my head somewhat violently against a spiral iron staircase in the old physiological laboratory. There followed months of extreme mental and bodily discomfort. Only those I think who have suffered from neurasthenia at its worst can properly appraise the misery involved. After six months, however, I completely recovered, and, so far as I know, have since been able to do as much as I should have done had this interlude been absent from my career.

My recovery was greatly helped by an event which I count as the most outstanding among my best gifts from fortune. I heard during my illness that Trinity College had made me a Fellow and elected me to a Praelectorship in Biochemistry. This same Praelectorship had in the past been allotted to various subjects and had been held by such distinguished men as Richard Jebb and Michael Foster. So far as the College itself is concerned the post carries no obligations. For me the election was salvation, and my debt to Trinity is great indeed. For twenty-six years I have received all the great privileges that membership of the College confers and have been asked for no single service in return. I know well that my election was in the first place due to the generous advocacy of Walter Fletcher. It is my hope that in any account of my career published after my departure the generosity of Trinity College will be emphasised.

In 1914 the University founded a Chair of Biochemistry and made me its first tenant, but my income as Professor was mainly derived from the Praelectorship in which Trinity allowed me to continue. A separate Department for the subject was provided in a part of the Physiological Laboratory in Corn Exchange Street, which was just then being vacated. Langley and his staff were moving into the new Drapers' Company's building.

Alas, the department of biochemistry had scarcely realised itself when came the war.

### *The First World War*

I have never ceased to wish that my war services could have been more real. When the war began I was, it is true, 53 years of age,

but not too old for some sort of active job. My clinical experience was, of course, too small, or by then too remote, for me to be of use as a doctor, and I was assured by many that as a laboratory worker I could be as useful at home as abroad. At any rate, no request for my help came from anywhere until W. B. Hardy started the Royal Society Food (War) Committee, of which I was a member, and for which my laboratory carried out investigations. We were also able to undertake, at Walter Fletcher's request, a certain amount of work for the Medical Research Council, of which I was one of the original members. Teaching for the Tripos continued at Cambridge, but in very depressing circumstances. It would, I think, have been well for me if I had been given a job away. From time to time during those years one was oppressed with a sense of futility, and this was demoralising.\* My wife, however, was meanwhile engaged upon real work of the right kind, and, though perhaps she did not realise it at the time, my pride in the great respect she won was a tonic possession.

### *The Twenties and the Thirties*

When the war was over the biochemical department soon made progress. Biochemistry was to become a separate subject for Part II of the Tripos and the research community was growing fast. Accommodation for research in the old Physiological Laboratory soon became inadequate and the University was unable to do anything to help in this difficulty. I knew that the Balfour Laboratory, where Girton and Newnham science students were taught before they were admitted to University classes, was at this time almost unused, and I found that both colleges were willing to accept the Biochemical Department as a tenant, though they could not afford to do so without the payment of a reasonable rent. How was money for this to be found? I was then, as later, a member of the board which advises the trustees of the Beit Research Fellowship Trust in the election of Fellows, and knew that the Trust Fund possessed an unallotted balance. With much hesitation I determined to see if help was here available.

\* Actually, Hopkins' services to the country during this period were far greater than would appear from the characteristically self-deprecating words of this paragraph. For long stretches the greater part of his time was spent in London on government work concerned with the country's food supply, and he used afterwards to describe with what anxiety the committee members used to watch the daily wall charts showing food imports, stocks, and consumption. Hopkins carried out also at least one mission in France of a nutritional advisory nature, in which his colleagues were Graham Lusk and Russell H. Chittenden from America. In later years he used to recall with amusement the very human trait which made Chittenden, who on scientific grounds advocated a protein diet decreased from the normal, adapt himself to the poor food of the time with greater difficulty than the other members of the Mission.

Through the kind offices of the secretary, Kingston Fowler, that help was indeed forthcoming. The trustees paid the rent of the Balfour Laboratory for some years, and there, in happy circumstances, our research community was housed.

In 1923-24, however, help for biochemistry came on a scale which surpassed our wildest dreams. It was then that the trustees of Sir William Dunn decided to devote no less than £210,000 to the development of the subject at Cambridge. In 1925 the present Institute was opened by Lord Balfour and I can claim that it has ever since housed a very active community. I can further claim, I think, that the community has been a happy one, for it and I have been fortunate in the possession of a staff which has always given loyal and unselfish help to its interests.

I will not attempt to deal with its history here, but I trust it may later be written. In any such history it must be made clear that but for the faith and enthusiasm of Walter Fletcher, to whom the Dunn Trustees went for advice, it might never have come into existence.

I suppose that most attempts at autobiography tend to take the form of an apologia. My own temptation has been to try and show that it is not altogether my own fault if I have remained—what I feel myself to be, compared with many who have received less recognition and fewer rewards—intellectually an amateur. I realise to-day that I know and have known no aspect of science *au fond*.

I have exaggerated no whit my lack of a basal education. In research I have at no time worked under or with an expert senior to myself. I know well, however, how much I owe to fortunate circumstances and to happy chance. I was led at a right moment to follow a path then trodden by very few and where each wayfarer was conspicuous. It is now a crowded path on which individuals cannot fail to jostle each other.

Looking back I wonder whether, had my training been more orthodox—had I come to Cambridge, for instance, in 1878 instead of 1898—I should not then have followed some more conventional career in which I might well have found it difficult to reach distinction.

To-day I find myself possessed of honours undreamt of.\* I can look back on a long life full of interest. I have enjoyed unalloyed domestic happiness and have a family in which I take pride. I enjoy good health at 76. *Fortunatus sum*.

1937

\* Knighthood, 1925; Copley Medal, 1926; joint Nobel Prize with Eijkman, 1929; Presidency of the Royal Society, 1931; Order of Merit, 1935.



# Sir F. G. Hopkins' Teaching and Scientific Influence

by

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## SIR F. G. HOPKINS' TEACHING AND SCIENTIFIC INFLUENCE

IN the evolution of biological knowledge, it has been said, the study of structure precedes that of function. This has been true not only at those high levels of organisation where anatomy preceded physiology but also at molecular levels where the structural organic chemistry of cell constituents was a necessary prelude to the study of what has come to be called dynamic biochemistry. During the nineteenth century the organic chemistry of animal and plant material occupied the attention of the biochemists of that period, largely though not entirely to the exclusion of the chemical behaviour of those same substances in the living organism. Such studies were carried out mainly in the great continental laboratories of chemistry, physiology and pharmacology; and the early studies on the sugars, proteins, lipoids and nucleic acids were done largely by German workers (e.g. Kossel, Salkowski, Hoppe-Seyler, Emil Fischer). At the close of the nineteenth century nothing of comparable importance had been achieved in Britain with the exception perhaps of the work of Thudichum on the cerebrosides, for which this country can hardly claim more than a portion of the credit.

The failure of British chemists to concern themselves with compounds of biological origin and importance rather than with synthetic achievement made the approach to biochemistry by way of organic chemistry, though successful on the Continent, impossible in England. Thus although Hopkins' tastes and training were those of a chemist, when he decided to turn to biological chemistry he felt compelled to enter the field through the portal of medicine. Physiologists were at least interested in the chemical changes occurring in the body and Hopkins' training as an analyst in a forensic laboratory, where he was accustomed to separating traces of foreign substances in tissues and biological fluids, gave him just that outlook and experience likely to be of immediate service to medicine. Thus his early studies showed the impact of the highly skilled analytical chemist on problems of physiological chemistry<sup>1,2,5,6,8,9\*</sup>; and it is clear that he impressed physiologists and medical men like Foster and Garrod with the value of his work, and that it was recognised that a new force had entered the field of physiology and medicine.

\* Numbered references are to the Bibliography.

His address to the Analysts<sup>26</sup> sets out what he thought were the shortcomings and needs of chemistry at this period as applied to medicine and allied subjects. He pleaded for better analysts capable of tackling the problems confronting physicians and physiologists, pointing out that the organic chemist of the period had ceased to interest himself in the animal and the plant as his predecessors of sixty years ago had done, and was, moreover, a poor analyst, incapable of affording much help to the physiologist and the physician.

But it was not in problems of structural and analytical chemistry alone that Hopkins was primarily interested. When he started his work the study of chemical changes occurring within the cell, as distinct from that of substances separated from it, was seldom attempted or even thought of; these happenings were wrapped around with mystery which, at that time, it was considered useless and even rather irreverent to try and penetrate. The chemistry of the cell was thought of as something different from laboratory chemistry; the substance of living matter was *protoplasm*; having entered this complex, molecules of food and oxygen were believed to lose their identity and to become incorporated in the living molecule or "biogen," where they lost their chemical characteristics and underwent mysterious and indefinable changes to reappear as end-products once more in a recognisable form such as urea and  $\text{CO}_2$ . Thus Sir Michael Foster had said:

The oxygen which enters the muscle from the blood is not involved in immediate oxidations, but is built into the substance of the muscle. It disappears into some protoplasmic complex . . . we cannot yet trace the steps taken by the oxygen from the moment it slips from the blood into the muscle substance to the moment when it issues united with the carbon as carbonic acid. The whole mystery of life lies hidden in that process and for the present we must be content with knowing the beginning and the end.

It is unlikely that Hopkins deliberately planned his early researches to prove that the protoplasmic myth was false, but consciously or unconsciously, the direction of his researches led to that result. His crystallisation of egg albumen and the care with which he demonstrated its fixity of composition went far towards removing the proteins—the principal constituents of "protoplasm"—from the category of indefinable substances to that of chemical compounds with a fixed composition.

As he went on he became increasingly impressed by the problem of chemical specificity in the animal organisation; and his approach

to dietetic problems arose from this angle. In his address to the Analysts<sup>26</sup> the problem is clearly stated; he emphasised that it was time that the energy and nitrogen value of diets should cease to be the sole criteria of food value; proteins must be considered from the viewpoint of their specific chemical character. This he illustrated by showing the inadequacy of zein and gelatin to maintain life and growth, and the importance of individual amino-acids like tryptophane.<sup>27</sup>

This led on to the question Hopkins had asked himself much earlier concerning the adequacy of pure proteins, fats, carbohydrates and minerals in animal diet, and culminated in the experiments leading to the discovery of vitamins.<sup>28</sup> In these studies it was not the facts only which were important, but the deep interpretation which Hopkins put on them. The conception of the cell as a chemical machine requiring units of special structure in order to function was something new and far removed from the vague notion of a protoplasmic complex within which occurred pseudo-chemical events, mysterious and undecipherable. Hopkins regarded the idea of protoplasm in so far as it was applied to biochemistry as mischievous and leading to obscurantism, and throughout his life he never missed an opportunity of attacking it. It was to him what "spontaneous generation" had been to Pasteur. As his work and thought developed his vision of the cell as a chemical machine took shape. In 1913 he made a comprehensive statement of his views in his presidential address to the Physiology Section of the British Association at Birmingham.<sup>29</sup> It would be folly to paraphrase or summarise this address since it is included *in extenso* in this volume\*; it is indeed a biochemical treatise in miniature and discloses fully and with amazing clarity Hopkins's inmost thoughts and speculations on the biochemistry of the cell. It is worth reading and re-reading; as one does so one finds oneself frequently referring to the title-page to verify the date, which one feels must be ten or twenty years later than the one recorded. It shows Hopkins at the height of his powers reviewing biochemical work from the days of Liebig onwards and interpreting it so as to build up a picture of the cell as the seat of ordered chemical events controlled in the interests of growth and function. As one reads it one feels it should have reached a wider public; maybe Hopkins meant that it should, for at that date he had promised to contribute a volume to the Biochemical Monographs, of which he and R. H. A. Plimmer were joint editors, and an expanded form of this address would have

\* See p. 136.

provided just what was needed; but a year later came the First World War, and the hoped-for monograph was never written.

On the continent a contemporary of Hopkins who was thinking along similar lines was Franz Hofmeister who, from 1885, occupied the Chair of Experimental Pharmacology at Strasbourg. Hofmeister was eleven years older than Hopkins and enjoyed from the start an uninterrupted career in academic science. As far as his scientific life was concerned therefore he was about twenty years Hopkins' senior, and the departments of the two men resembled each other in being concerned with a great variety of problems. Hofmeister himself was interested in the chemical constitution of the cell in relation to function; in a lecture published in 1901 entitled "Die chemische Organisation der Zelle" he considered the cell as a machine for the transformation of energy, repudiating the well-worn analogy with the steam engine on the ground that in the latter the material of which the machine was constructed was unimportant whereas in the former the material ("protoplasm") itself provided the machinery by means of which the energy transfer occurred. Hofmeister was not, however, a subscriber to the "protoplasm" view of biochemistry, and pointed out, *inter alia*, that the small size of the cell was no bar to the conception that colloidal ferment molecules were the agents by which chemical change in the cell was brought about, calculating that within, for example, the liver cell there was ample space for many millions of such colloidal molecules. Hofmeister also discussed the interrelation of chemical and morphological differentiation in embryonic life and postulated an orderly procession of enzymes appearing each from its precursor as development proceeds. He also speculated on the function during life of the colloidal substrate in localising different chemical reactions which would otherwise antagonise each other. "An ordered sequence of chemical reactions in the cell presupposes the work of individual chemical agents and a definite movement of the products, in short a chemical organisation. . . . If the morphologist on the one hand investigates the protoplasmic structure, and the biochemist on the other hand tries to assess the chemical processes of the same protoplasm with his cruder but deeper methods, it amounts in the end to only two different approaches to the same problem." It is clear from this paper alone how similar were the ideas of the two men; if they ever met it was very briefly, but Professor E. Friedmann, who worked in Hofmeister's department from 1900 to 1908, and in Hopkins' laboratory from 1930 to 1946, testifies that the work of each was well known and valued by the other. Hofmeister did not long survive the First

World War; his last paper was published from Würzburg in 1922, at the age of 72, twenty-four years before the death of Hopkins.

This time-interval gave Hopkins the advantage, so far as cell chemistry was concerned, of the experimenter over the speculator. Hofmeister worked before the period of intensive enzyme study which occurred from about 1920 onwards. The nature, function and interaction of intracellular enzymes, which Hofmeister apprehended intuitively, became in Hopkins' laboratory subjects of direct experimental study. It was, indeed, fortunate that Hopkins' gifts and faculties continued unimpaired to such a ripe age and more than compensated him and us for the disadvantages of a late start and for the interruption of the First World War.

As the years passed Hopkins' teaching progressed along the lines of the Birmingham address, and it is remarkable how the development of biochemical knowledge followed the course he had foreshadowed. More and more was he impressed with the importance of chemical specificity as shown by the demand of the cell for certain molecules and its inability to replace them by even closely related substitutes, but particularly he was absorbed by the study of the problem of specific catalysis and its implications. Here he found the satisfactory substitute for "biogen," or the molecule of protoplasm, which from start to finish he never tired of repudiating. He believed that in the possession of specific catalysis lay the difference between living and non-living material,<sup>85,88</sup> and permitted himself to speculate on its relation to the origin of life. In 1933, twenty years after his Birmingham address, he was again talking to the British Association, this time at Leicester,<sup>93</sup> and, after giving the giant molecule one more castigation, he proceeded to develop the theme of the indispensability of specific enzyme catalysis to life as we know it. He emphasised the adequacy of biochemistry to throw light on such subjects as the mechanism of heredity and development; he even touched very lightly on the psycho-physical problem and the nature of the material systems associated with consciousness, but he did not venture to discuss this problem. Nevertheless, it occupied his thoughts, and the writer recalls that he once remarked that the transition of the non-living to the living presented to his mind less difficulty than the origin of consciousness, remarking that our difficulty was greater because we do not know at what level consciousness starts; "what we want," he said whimsically, "is a colour test for consciousness."

Hopkins' teaching as shown in his addresses is well represented in this volume, and one must now turn to his direct influence on his pupils.

As a lecturer Hopkins was definitely for the advanced research worker rather than for the elementary student; also he could strike the spark. The writer well remembers in 1905 or 1906 when he substituted for the Professor in an elementary lecture; he talked about lactic acid, muscular contraction and, though much that he said was speculative (and possibly incorrect in the light of modern knowledge) it opened a new world of thought which the didactic lectures previously handed out to us had never even hinted at.

In his early years at Cambridge he developed physiology as part of the advanced course of physiology for medical students and his early pupils testify to the inspiration of them, Edith Willcock, now Mrs. Stanley G. Willcock, an impression left on her mind after each of Hopkins' lectures was a statement of information than a realisation of new unexplored tracts and the unfolding of important research. His power of evoking enthusiasm was shown by another of his early pupils, Sir Edward Mellor, "how, whilst still a student he (Hopkins) brought me a state of enthusiasm sufficient to make me devote my attention to this problem for a fortnight, to work, in order to find a method to decide whether the rate was one-thirteenth or one-twentieth of that of the department grew larger the organisation was more deputed to others, and by the time he had attained the status of a Part II class of its own all senior students shared in the work. The Professor was present in the advanced classroom and his approach to the discussion of failures stimulated and encouraged equally.

In 1935 biochemistry was introduced into the Tripos; this move occurred in spite of the opposition of some members of the biological department that the subject was moving too quickly for teaching; Hopkins, however, was of the opinion that it was so important and of such value that no science student in Cambridge should be without of acquiring an elementary knowledge of it, and he carried the day.

\* Hopkins Memorial Lecture, *Journ.*

the disappearance of biochemistry from the elementary curriculum otherwise than with dismay.

It was, however, in the research department that Hopkins' influence was greatest. He never sought to develop a school, as did many of his contemporaries on the Continent, where a whole laboratory might be working on aspects of the professorial problem by the professorial methods. Hopkins frequently suggested problems connected with his own work to his junior workers, especially to those who came to him from overseas, but he usually contented himself with indicating the lines to be followed, leaving them to worry out the details. But to *all* members of the research department he was ever available for consultation and advice. Newcomers quickly lost their awe of him and learnt to drift into his room and, in the opinion of all the rest, to waste his time; it was, in fact, the opinion of each member of his department that everyone else traded on the Professor's good nature and woefully wasted his time. But it was through these endless and informal talks that Hopkins put his ideas across. Every newcomer quickly learnt that he was expected to think as well as to work, and was encouraged to do so by the discovery that his efforts in that direction appeared to be of value and even of help to the Professor.

At weekly tea club meetings each member of the department in turn gave a paper on his own work, either while this was still in progress or just completed. It was here that Hopkins was quite unequalled. Oftentimes when the material seemed dull and unimportant the old hands used to wonder what the chairman would find to say. Never was he known to fail; by skilful suggestions and questions he turned the most unpromising material into something interesting and significant, leaving the author encouraged and sufficiently self-confident to meet the more obvious criticisms of his colleagues. On looking back it seems likely that this success was due to the fact that Hopkins alone amongst us had a clear mental picture of the cell as a biochemical machine and into this scheme was able to fit what seemed to the rest of us mere isolated observations, thus giving them significance.

The Professor lectured once a week at 4.30 p.m. to his advanced class, and the whole department attended as a matter of course. These lectures were often reminiscent and historical and invariably stimulating and suggestive. They often bore little evidence of previous preparation and the theme was developed as the lecture proceeded; when the Professor was talking in this way he generally shut his eyes very tight.

In these lectures the giant molecule came in for its share of castigation and was a source of amusement to the irreverent members of the audience. This "inhibitor of thought" had never worried them and they could not see why it should worry him; it was, in fact, a laboratory joke, and when the journal *Protoplasma* first appeared, in 1927, the members of the department professed to wonder whether, on account of its unfortunate title, it would be allowed a place in the departmental library.

Hopkins delighted to foster in his department lines of work far removed from his own personal studies. Bacterial chemistry, actually first introduced into the department about 1902 by himself and Cole,<sup>25</sup> in their classical studies on the constitution of tryptophane, was revived by him in 1917, when Harold Raistrick came from Leeds and was joined by Barbara Clark to inaugurate work on this subject supported by the Medical Research Council; this has been maintained and extended till it has to-day developed into a sub-department. In a similar way chemical embryology was developed by Joseph Needham, who formed a small school working on this subject till interrupted by World War II. Invertebrate and comparative biochemistry, inaugurated by Joseph Needham and subsequently developed by Ernest Baldwin, is still a feature of both research and teaching in the department. Plant biochemistry was very early introduced into the department by Muriel Wheldale (Mrs. Huia Onslow), and is now an important group activity directed by Robin Hill.

Biological oxidations and enzyme chemistry, now in the hands of Malcolm Dixon, form perhaps the corner stone of biochemical work in Cambridge and, like muscle chemistry, developed by Dorothy Needham, are directly descended from Hopkins' early studies.

In trying to evaluate Hopkins' unique contribution to biochemistry it may perhaps be said that he alone amongst his contemporaries succeeded in *formulating* the subject. Among others whose several achievements in their own fields may have surpassed his, no one has ever attempted to unify and correlate biochemical knowledge so as to form a comprehensible picture of the biochemical machinery of the cell and its relation to life, reproduction and function. Hopkins' picture of cell life as an ordered sequence of events governed by specific catalysts is daily being verified, particularly in studies on unicellular organisms. Here the processes of both anabolism and catabolism have now been shown to occur in ordered steps, each controlled by its specific catalyst, the production of which is in its turn shown to be governed by an element of the nuclear structure



IN THE LABORATORY ABOUT 1941.

"A very distinguished organic chemist, long since dead, said to me in the late eighties, 'The chemistry of the living? That is the chemistry of *protoplasm*; that is super-chemistry; seek, my young friend, other ambitions!' . . ." (p. 245).



and may be lost in artificial or natural mutations. If such a loss occurs in the anabolic chain of events the cell becomes incapable of development unless the product of the action of the missing enzyme is supplied in the food of the cell. If the lost enzyme catalyses some step in catabolism, some unusual product of metabolic breakdown may appear. Owing to the capacity of the microbial cell to adapt itself to changing conditions it provides material specially suitable to illustrate the ordered sequence of events visualised by Hopkins. Similar interference with animal or plant cells frequently has consequences fatal to continued life. The microbe "makes do" and finds a way round, and in so doing provides a clue to the natural sequence which has been interrupted.

When these same ordered sequences have been disclosed they seem to hold with surprising uniformity throughout the biological world; tryptophane, for example, is built up by the same steps in the animal and in the microbe, and plays some indispensable part in all living organisms, as Hopkins & Willcock, in 1906,<sup>30</sup> showed in the case of the rat.

Hopkins started working at a time when the methods and aims of biochemistry needed to be apprehended and stated before the subject could emerge as a distinct science with a field and methodology of its own. Under his influence the subject developed along the lines he envisaged, not because he so envisaged them, but because his view of this aspect of nature was the true one. There comes a time in the life of every science when it requires a midwife before it can emerge and start an independent existence. What Lyell did for geology and Claude Bernard for physiology Hopkins did for biochemistry. It is a matter for deep regret that he left behind no single book embodying his conception of the subject he brought to life. His views have, therefore, to be assembled from lectures, addresses and publications belonging to various periods of his life and witnessing to his thought and teaching.

It need not be supposed that Hopkins achieved all that he did by lying on a bed of roses. He was personally beloved, but in establishing biochemistry he met, at times, with considerable opposition and criticism from the exponents of subjects upon the confines of which it bordered, and from non-scientists. When the time came, however, he never shrank from applying the necessary pressure, and his many friends stood by ready to help. It is, moreover, noteworthy, as illustrating the slow growth of appreciation of his science, that at the time of his death only one other university in England, and

one in Scotland, had established biochemistry as a subject for a degree, or attempted to share with Cambridge the task of training the biochemists of the future.

In 1925, the year in which Hopkins received his knighthood, there appeared, in the illustrated journal of his laboratory, an excellent picture of our "verray parfit gentil knight" on a war horse in a full suit of armour bearing a pennon inscribed with the motto "Gluthy Owne"\*; the topical allusion is obvious, but the years have brought it a deeper meaning. In his early life Hopkins had felt himself capable of achieving a work for science in establishing biochemistry as a separate study and in stamping it with his own mark. He fulfilled this dream of his youth by thought, work and struggle, showing a long-continuing courage and tenacity of purpose concealed from the superficial observer by his gentle, slightly hesitating, courteous manner.

\* Reproduced on p. 326.

A Catena of Excerpts from the Scientific  
Papers of Sir Frederick Gowland Hopkins

with commentary

by

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# A CATENA OF EXCERPTS FROM THE SCIENTIFIC PAPERS OF SIR FREDERICK GOWLAND HOPKINS

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## INTRODUCTION

IT was once my lot to be asked to prepare a review on modern influences in nutritional science. I realised at the outset that preparatory to this I would have to undertake a task I had too long neglected—to make myself more familiar with the many publications and addresses of Hopkins himself on nutritional and allied subjects. This necessitated a certain amount of research; a bibliographical list had to be compiled with the aid of abstract journals, and a collection of the originals brought together from a wide variety of sources and covering a period of a good many years. At one stage I ventured to seek the aid of the Hamlet of the piece, only to discover that, characteristically enough, he possessed neither a collection of reprints nor even a list of his own papers. As the reading proceeded, I came to feel that others might like to share in it. Rather than try to paraphrase the original text, it has seemed better to hear Hopkins speak

in his own highly characteristic phrases. My object has therefore been merely to provide a connecting link between the separate quotations.

In reading these papers one is conscious of what can only be called a "Hopkins atmosphere". In the presentation of his scientific results science becomes united with art. No bald statement here in technical jargon of the bare facts of the case. One can feel rather the anxiety of the writer that his results should be presented in such a way as to capture the interest and imagination of the reader; the problem, and the development of the theme, turn into a romantic adventure, and the reader is carried along not only by the unfolding of the narrative but equally by the richness and variety of the vocabulary. In the persistent choice of the *mot juste*, the strong emphasis, and the achievement of a balanced sentence, one is reminded of no writer so much as Macaulay. These attributes are surely to-day becoming a disappearing quality—in that "cast iron" presentation of material which is often required by scientific periodicals.

The papers in question cover so wide a variety of topics—ranging from butterflies to vitamins, proteins to uric acid, xanthine oxidase to glutathione—that it is easy to overlook a certain underlying logical sequence which may be detected in the succession of research problems. A youth's interest in butterflies combined with an apprenticeship to a public analyst lead to a chemical study of the pigment in the butterfly's wing. This proves to be a derivative of uric acid. In the course of this work methods suggest themselves for improving the technique of the uric acid determination. Interest in uric acid then focusses attention on other excretory functions and especially on the supposed influence of dietary protein on the uric acid output. Studies on proteins accordingly follow, not least on the dietary values of proteins. From this it is an inevitable step—experimenting with purified proteins and artificial dietaries—to find that certain "accessory factors" cannot be overlooked. But in the meantime, the geographical contiguity of a pioneer biochemist and skilled analyst, on the one hand, with a leading expert on muscle physiology on the other, brings about a happy association in the laboratory which results in the clarification of another important field. Resuming the main thread: in searching for accessory factors attention is given to the sulphur-containing substances in food\*, and so the sulphydryl reaction is traced to its source—glutathione is isolated. That brings one to the mechanism of biological oxidations and so to the last phase of Hopkins' work.

\* Vitamin B<sub>1</sub> has since justified the intuition.

Adopting this roughly chronological classification, we shall now proceed to quote section by section.

## I. BUTTERFLIES AND URIC ACID

The first entry<sup>1</sup> takes us back close over seventy years—to an issue of *The Entomologist* for November, 1878. This journal contains on its title page the motif:

By mutual confidence and mutual aid  
Great deeds are done and great discoveries made.  
POPE'S *Iliad*.

Within, one finds a section headed "Entomological notes, captures, &c.," consisting of contributions sent in by readers from different parts of the country describing their finds, or raising various queries for answer by the editor. The twenty-fifth out of a series of twenty-eight such notes may be cited in full:

*Brachinus crepitans*.—I have observed that the little bombardier beetle has been exceedingly plentiful this year, and I feel interested to know if this has been the experience of others. I caught my first specimen in March, and this was the first I had ever seen here; since then, and till quite lately, they have appeared in great numbers. On the South Downs, near Eastbourne, I also saw several of these insects, though I have no recollection of having observed them there before. Altogether *Brachinus* seems to have been an exception to the general scarcity of his order this year. It is a very sociable insect, and I have seldom seen one without finding others close by. The beetles are very partial to my sugar compound, and have swarmed on trees prepared for moths. *Colias edusa* has quite disappeared from here this year.—F. G. Hopkins, Ridgeway, Enfield.

Fifty-seven years later, *The London Naturalist* for 1935<sup>109</sup> contains the address\* of Professor Sir F. Gowland Hopkins, O.M., M.D., F.R.C.P., P.R.S., upon the occasion of his election as Honorary President of the London Natural History Society in succession to Lord Grey of Falloden:

I will here venture, hoping for your forbearance, to intrude a fragment of personal history into my remarks. Like Bacot himself and, I suspect, like very many of my audience, I was in my early days an ardent collector of butterflies and moths and (next easiest, I think, for the boy or the amateur) of beetles. It was one day in March of the year 1878—it is just 57 years ago—that I first made, with infinite pleasure, the acquaintance of the little bombardier beetle, *Brachinus crepitans*, if that be still its accepted scientific

\* Reproduced in full p. 269, Bibliography No. 109,

designation. It was plentiful that year and I found it in a northern suburb of London and on the South Downs near Eastbourne. Now, when one day I beheld, without previous knowledge of their abilities, how these insects on being disturbed eject a violet vapour into the air, a most effective act for offence or defence, I felt an intense curiosity to know not only how this volatile stuff could be made and stored but in particular what the stuff could be. I tried a few experiments, putting one bombardier after another into the same test tube, and encouraging each one to shoot. The vapour condensed on the side of the covered tube and I thus collected a little of the material. It was very little, however, and I was a youth with no chemical training, so nothing came of my researches. I think, however, that from that time my fate was sealed. Though the designation was not yet invented, I became there and then a biochemist at heart.

I am reminded by a friend of a circumstance that I had quite forgotten, namely, that in the volume of the *Entomologist* for 1878 is published a note of mine on the capture of these beetles, with remarks upon the frequency of their occurrence in the Spring of that year. This was my first scientific publication. I find it contained no account of my experiments, but this I think was due to the shyness of youth.

After *The Entomologist* of 1878 a gap of eleven years follows before the appearance of the next contribution,<sup>2</sup> to be found in the *Proceedings of the Chemical Society* for 1889. The author, in the meantime, had been articled to a public analyst, as described in his Autobiography.\*

The specialised technique of the analyst is now able to serve the curiosity of the naturalist.

For the most part the colour effects on the wings of lepidopterous insects are probably due to purely physical causes, and not to the presence of any definite pigment. But in some cases pigments are undoubtedly present. They are for the most part very difficult to isolate, but an exception to the rule occurs in the case of a certain yellow pigment which is found in its purest form in the common English brimstone butterfly. The pigment may also be detected in the wings of a very large number of day-flying lepidoptera. It may be obtained from the wings by simple treatment with hot water. . . .

\* Recollections of the work of the Analyst's Department at the Home Office are contained in the obituary notice<sup>31</sup> of Sir Thomas Stevenson, in *The Analyst* of 1908. Sir Thomas was appointed "senior scientific analyst to the Home Office" in 1882:

"When I went [says F.G.H.] to him in 1883—joining Bodmer, who had already been his assistant for five years and was to remain with him for ten years more—the laboratory was still full of memories of the famous Lamson case of poisoning by aconitine, the investigation of which Stevenson, in conjunction with Dupré, had conducted with extraordinary skill a few months before.

"There is no need to discuss here the many sensational trials in which Stevenson's evidence played a part. Some of them, such as the Moat Farm case, the Maybrick case, the cases of Neil Cream and Chapman, the St. Neots murder, and many others, will linger in the public memory. . . ."

Details of the separation of the pigmented material follow: it yields a murexide reaction, and in its properties it agrees closely with "mycomelic acid," a well-known yellow derivative of uric acid, to which it is probably closely allied; perhaps a condensation product of uric and mycomelic acids. The author "hopes to obtain a sufficiency for accurate ultimate analysis." After the lapse of a couple of years, a further short note appears in *Nature* for 16th December, 1891,<sup>5</sup> contributed from the Sir William Gull Research Laboratory, Guy's Hospital.

I have ventured to call it [the pigment] lepidotic acid. . . . In my original paper I ventured to suggest a formula. . . . I am looking forward to the appearance of Mr. Beddard's book. The literature of the subject of animal coloration is not easily accessible and a text book thereon will be a valuable acquisition. We have, it is true, the interesting work of Mr. Poulton, but the subject is there treated from what is, perhaps, a somewhat limited standpoint.

Not until 1896 did the full details of the complete research appear in the *Philosophical Transactions* of the Royal Society ("by Mr. F. G. Hopkins, M.B., B.Sc., Demonstrator of Physiology and formerly Gull Research Student, Guy's Hospital—communicated by Professor E. Ray Lankester, F.R.S.").<sup>6</sup> It is concluded that the cause of the opaque whiteness in the wings of Pieridae is uric acid. In the allied yellow insects the pigment is a related substance "probably identical with a substance obtained when uric acid is heated with water under pressure . . . the mycomelic acid of Hlasiwetz."

The yellow wing pigment is frequently found in material voided from the rectum of a Pierid, showing that in the coloured insects as well as in the white a normal excretory product subserves the purposes of ornament.

Many different varieties of insects are examined, and the investigation shows a combination of the skilled procedure of the chemist with the specialised technical knowledge of the entomologist. It is interesting to notice that in the acknowledgements, Dr. William Bateson, of St. John's College, Cambridge, is thanked for contributing from his stores of insects, and Professor Raphael Meldola for advice on chemical matters. Spectroscopic measurements were "obtained with a spectroscope belonging to Dr. A. E. Garrod."

Looking back, in 1935, in the address already mentioned,<sup>109</sup> Hopkins comments on this work.

. . . I myself, a few years after I had made the acquaintance of *Brachinus* and his ammunition, became interested in the pigments of lepidoptera. In

the early eighties I noticed that the wing pigments of coloured Pierids were soluble in water and felt that this gave one a chance of discovering their nature. To make a long story short, I became early convinced that these pigments were derivatives of uric acid, while the wings of white Pierids contained uric acid itself. I had thus come across a case of the use of excretory substances in ornament, a phenomenon which may shock or please the aesthetic sense according to the point of view. . . .

I will here, by the way, just mention in parenthesis that my work on the pigments of the Pieridae has suffered, forty years after it was published, a correction which had I been younger might have seemed to me a tragedy, but now I can bear it with equanimity. These pigments, yellow and red, are sure enough uric acid derivatives, but the parent material in the scales of the white species is not, as I thought, uric acid itself but a related substance with very similar properties not hitherto known to chemists. The distinguished organic chemist Heinrich Wieland, who has made this correction, admits that for long he himself believed the substance to be uric acid, and it was, *inter alia*, the fortunate application of a discriminative qualitative test, non-existent in my time, which first led him to the distinction, so that I do not suffer greatly in pride over this happening. . . .

## II. URIC ACID AND EXCRETORY PROBLEMS

The principle of Hopkins' process for the estimation of uric acid is thus described in his paper in the *Proceedings of the Royal Society* ("by Mr. F. Gowland Hopkins, B.Sc., Gull Research Student at Guy's Hospital—communicated by Dr. Pye Smith, F.R.S.")<sup>6</sup>:

It has long been known that ammonia and salts of ammonia will after long standing completely precipitate the uric acid. . . . Upon this observation the well known Fokker-Salkowski process is based.

But the crucial fact that *saturation* with the ammonium chloride renders the separation rapid and absolutely complete does not seem to have been previously observed.

It is added that for rapid clinical work titration with  $\text{KMnO}_4$  may be used for the final determination of the liberated uric acid, following the procedure of Sutton. An earlier note in *Guy's Hospital Reports* for 1891<sup>4</sup> gives technical analytical details—the method proves "its absolute accuracy" and "can be confidently recommended." A final contribution on the subject, in the *Journal of Pathology and Bacteriology*, adds:

After using the method for more than a year, with a large number of normal and pathological urines, I am strengthened in my belief that it is a process of very great accuracy. By its means uric acid may be estimated with an error of less than 1 per cent.; a degree of accuracy unattainable in

the case of any other organic constituent of urine. . . . A saturated solution of ammonium chloride is, in fact, a menstruum in which ammonium urate is absolutely insoluble.

In the preceding section of these notes passing mention was made of the spectroscopic interests of A. E. Garrod, then physician at Guy's, and later to be Regius Professor of Medicine at Oxford. The association with Hopkins at this time leads to an interlude of a series of three papers in which spectroscopic technique is further applied to problems relating to urinary excretion. The first<sup>10</sup> deals with the interesting phenomenon of excretion of haematoporphyrin in patients taking sulphonal:

The chemical and spectroscopic character of the dark red urine of sulphonal poisoning have been carefully studied by Salkowski, Hammarsten, Stockiss and MacMunn, and our object in bringing forward the following three cases which have come under our notice is to emphasise certain points to which these eminent observers have already called attention, and in certain particulars to supplement their results.

The two other papers with Garrod deal with urobilin.<sup>11,14</sup>

The earliest practical outcome of the application of the spectroscope to the study of the colouring matter of urine was the discovery by Jaffé of urobilin . . . the most extensively studied of the urinary pigments.

Suggestions had been made that there were two distinct forms of urobilin, but Garrod and Hopkins conclude that "the urobilin of normal urine is identical with that present under a variety of morbid conditions."<sup>11</sup> The results of elementary analyses of urinary and faecal urobilin "confirm the conclusion that . . . faecal and urinary urobilin are identical in their elementary composition."<sup>14</sup>

By the end of the century, Hopkins had established his position as the principal British expert on the chemistry of the urine. In a new *Textbook of Physiology*, which appeared in 1898, under the editorship of E. H. Schäfer, Professor of Physiology at University College, London, it is interesting to note the names of the eminent contributors, including Professor A. Gamgee, Professor W. H. Gaskell, Professor F. Gotch, Professor W. D. Halliburton, Professor J. N. Langley, Professor J. G. M'Kendrick, Professor B. Moore, Professor J. Burdon Sanderson, Professor C. S. Sherrington and Dr. E. H. Starling. F. Gowland Hopkins, B.Sc., M.B., "Demonstrator of Chemical Physiology in Guy's Hospital Medical School" is the author of the chapter on "The Chemistry of Urine."<sup>18</sup> This remains perhaps his most considerable effort towards the writing of a student's text, or

comprehensive review. It covers 68 pages, and embraces the following topics: "Quantitative composition of urine; variations in its amount and specific gravity; its chemical reaction; the nitrogenous constituents; total nitrogen, urea, ammonia, uric acid, xanthin bases, creatinin, hippuric acid; amido-acids; proteids; the aromatic substances; the carbohydrates; glycuronic acid and its conjugated compounds; oxalic acid; acids and oxyacids of the fatty series; colour of the urine and the chemistry of its pigments: the preformed pigments of normal urine, chromogenic substances, the pigmentation of pathological urine; the inorganic constituents; general characteristics of the organic urinary compounds; comparative chemistry of the urine."

Hopkins' philosophical outlook on what, to others, would seem a somewhat arid technical subject is apparent from his opening sentences:

*General considerations.*—The chemical study of the urine gains its chief importance from the light which it throws upon the processes of metabolism. It is concerned mainly with a consideration of the nature and amount of the various metabolic end-products, normal or pathological, which converge into and appear together in the highly complex excretion of the kidneys.

The great importance of this point of view has led to perhaps undue neglect of a second aspect of the subject—the consideration of the renal excretion as a complex whole; as a chemical fluid with individual characters of its own; characters which are not to be foretold from a knowledge of the nature and amount of each constituent considered separately, but require for their explanation the further consideration of the mutual effects of the constituents one upon another, as they exist side by side in solution.

This study of the properties of the urine as a whole must be pursued if we are to understand with exactness the nature of the processes which go on in the kidney, and if we wish to interpret aright the ultimate behaviour of any given type of urine while in the urinary passages, or after it has left the body.

But while the first-mentioned line of study requires in the main the services only of analysis—the earliest and best understood of the weapons of chemistry—the second depends upon our more recently won, and as yet very incomplete, knowledge of chemical statistics, and of the conditions of equilibrium in salt solutions. . . .

### *Urinary Excretion and Dietary Protein*

Interest in the urinary excretives now begins to become diverted more towards a study of their relation to dietetic influences. Leading in this direction we find first a note "by W. Hale White, M.D., and F. Gowland Hopkins, M.B., of Guy's Hospital," in the *Journal of*

*Physiology*, Vol. 24 (1899), on the excretion of phosphorus and nitrogen in a case of leucaemia.<sup>21</sup> Milroy and Malcolm had reported that the amount of phosphorus in the urine was lowered in leucaemia, and had suggested that this probably indicated a diminished breakdown of the leucocytes rather than an increased production. White & Hopkins found no departure from normality in the P and N excretion in the case they examined; but the more significant portion of their paper is a discussion of the breakdown of nucleins, by which  $P_2O_5$  and alloxuric acid are produced.

A more important development, however, is a contribution by Hopkins & Hope,<sup>19</sup> "on the relation of uric acid excretion to diet," which deals with the question—"Are nucleo-proteids the chief dietetic precursors of uric acid? A criticism of the conclusions drawn from thymus feeding." Summarising their results in a paper<sup>20</sup> read at the Fourth International Physiological Congress at Cambridge in August, 1898, Hopkins & Hope write:

The authors have confirmed the statement of Mares that after a meal the increase of uric acid in the urine is immediate and has a duration shorter than that of the increase of urea. They call attention to the difficulty of reconciling this fact with an origin from nucleins, which are unaffected by the earlier (gastric) period of digestion.

Since the ingestion of pure nuclein, or of a pepsin hydrochloric-acid extract of the thymus gave no increase in the excretion of uric acid:

the ascription of all uric acid production in the mammal to the breakdown of nucleins is over hasty.

The fuller account, in the *Journal of Physiology*,<sup>19</sup> contains the following passage, which, incidentally, furnishes us with a good example of Hopkins' writing:

The belief that uric acid has this special origin (off the lines, so to speak, of general metabolism) predisposes to the acceptance of statements like those of Mares, which ascribe to it a special period and rate of excretion, independent of the main nitrogenous excretory tide. This is probably why the otherwise striking results obtained by this observer have not been submitted to the test of repetition. . . .

There can be no doubt, after consideration of the available experimental evidence, that the degree in which uric acid production is influenced by diet, depends very directly upon the nature of the ingesta. The marked difference found by all observers in the effect of eggs and milk on the one hand, and of muscle, or especially of thymus gland, on the other, together with the absence of any increase after non-nitrogenous diet is

sufficient proof of this. . . . At the same time we believe that the experimental evidence we have brought forward should lead to a revision of the prevalent view that the difference is due entirely to the varying proportion of nucleoproteids which these dietaries contain. . . .

The chief evidence for the view that nucleins play a predominant rôle as uric acid precursors is based upon the results of thymus-feeding. Experiments detailed in the paper show that extracts may be prepared from this gland which contain at most traces of nucleins or nucleic acid, but which when ingested produce the characteristically large excretion of uric acid.

It is therefore suggested that of the total quantity of uric acid normally excreted, that portion which bears a more immediate relation to food does not arise from nucleins but from some more soluble constituent of the diet acting either as a direct precursor or as a factor in a synthetic process.

The probable origin of the uric acid which is so rapidly excreted after a meal we hope to discuss in a future paper.

### III. PROTEIN CHEMISTRY

Simultaneously with these studies of the dietary rôle of protein—which were to be continued later from the more specific angle of the rôle of the “essential qualitative factors in nutrition”—a series of papers was also appearing dealing with the more purely chemical properties of the proteins. These include such well-known contributions as those on the halogen derivatives of proteins, on the crystallisation of proteins, and on the isolation of the amino-acid tryptophane.

#### *Halogen Derivatives from Proteins*

Describing the origin of their observations on the halogen derivatives of proteins studied by Hopkins, first with Brook and then with Pinkus<sup>12,15</sup>, Hopkins & Brook write<sup>13</sup>:

A chance observation made last autumn of the efficacy of bromine as a precipitant for urinary albumen led us to the experiments described in this paper.”

The story proceeds with the account of how they found that “various proteins were completely precipitated by the direct action of chlorine, bromine, or (under somewhat modified circumstances) of iodine.” The materials resulting were apparently “different from the haloid peptone salts of Paal.” “They contain a *strikingly large* percentage of halogen.”

The following passages from this paper may be quoted, not least because of their characteristic style of presentation:

It will be held unlikely that halogen derivatives with chemical individuality in the strictest sense, can be precipitated by comparatively simple

mean, from such a substance as egg-albumen—for which, itself, it would be bold to suggest individuality. But it is not possible that the congruence of analytical data, obtainable even in a preliminary and merely tentative study such as is embodied in this paper, can be without significance. . .

We have at present made no serious attempt to obtain the products in crystalline form. Their general behaviour indicates that they will in probability crystallise with no more readiness than does the original proteid. This is perhaps no matter for grave regret. The brilliant work of Schmiedeberg and Dieckhoff, and the ingenious expedients of many who have followed them, have given us a series of crystalline proteids, but it cannot be said that the possession of this material has hitherto greatly influenced progress towards a knowledge of the protein molecule.

Much more copious is the observation, not yet chronicled in this paper . . . indicating lines of work, to which we hope to contribute our share in the future.

This paper is preceded by an introductory historical note, referring to work on the subject recently published by other authors. In this, priority is conceded to "Blum of Frankfurt-on-Main for the application of artificial haloid proteid products to therapeutic use":

The physiological and therapeutic results which he claims to have obtained are sufficiently striking; the substances serving, in fact, exactly such purposes *as require production might suggest*. In the very number of the *Revue* which contains the summary referred to above appears a paper by Liebricht. . . [Reference follows to the earlier contributions of Lépine, and to the authors of a paper in the contemporary issue of *The Analyst*.] To them however belongs the credit of having unearthed from the early literature a reference to previous observations which had escaped us. As far back as 1840 Mulder described the chlorination of proteids by direct action of the halogens. . . it is clear that actual priority upon the chemical side of this subject belongs to Mulder, and to him alone.

Our own work, however, traverses the ground of no other observer and we feel justified in publishing the paper exactly as it was originally written.

### *Crystallisation of Proteins*

"The ingenious expedients" for obtaining crystalline proteins referred to in one of the passages just quoted were shortly to be further improved by Hopkins & Pinkus themselves.<sup>16,17,22</sup> In the *Proceedings of the Fourth International Physiological Congress* the principle of their method is concisely summarised in these words<sup>16</sup>:

A modification of Hofmeister's method of crystallising egg albumen.—When egg white is treated with an equal bulk of saturated ammonium sulphate solution after the method of Hofmeister, abundant ammonia is always

liberated and the difficulty experienced in obtaining the first crop of crystals is apparently due to the associated alkalinity of the mixture.

If, after filtering off the mucoids, etc., the filtrate be rendered just faintly turbid by further addition of ammonium sulphate and if then sufficient dilute (10 per cent.) acetic acid be added to produce a slight permanent precipitate, the proteid will rapidly crystallise without evaporation. Usually abundant crystals are obtained in the course of four or five hours. Although the precipitate originally produced by the acetic acid is amorphous, this also rapidly changes to a crystalline form, and the final result is a large yield of needles quite unmixed with globules or amorphous material.

The fuller report in the *Journal of Physiology*<sup>17</sup> takes the form of an eminently readable essay:

We believe it is generally found that the preparation of crystalline egg-albumin by Hofmeister's method is a matter of some difficulty. . . .

We have been able to make a modification in the process which, in itself of quite a minor character, has proved of the utmost importance in promoting the ease and rapidity of crystallisation and in increasing the proportionate yield of the product. By introducing this slight modification we are able in fact to obtain large quantities of finely crystalline egg-proteid with great certainty, and in the course of twenty-four hours from the commencement of the operation. . . .

It is generally considered necessary that perfectly fresh eggs should be used for the purpose of crystallisation. We have found those ordinarily to be bought at the shops as new-laid give perfectly reliable results; and, although the degree of freshness undoubtedly affects the ease with which crystals are to be obtained, we have secured quite satisfactory preparations from the cheapest eggs in the market, rejecting, of course, such as are obviously decomposed.

In the third paper on the subject,<sup>22</sup> the romance inherent in scientific research is again made fully apparent; but perhaps the most important point is the stress on the *chemical individuality* of the isolated protein:

The chemical study of animal proteids made one of its greatest advances when Hofmeister showed that under special conditions egg-albumins could be crystallised. When to this discovery was added the observation of Gürber showing that Hofmeister's method of crystallisation could be extended to certain serum-albumins, there became available for research representative proteid material obtainable in practicable quantities, and possessing some guarantee of purity.

The work of the decade which has elapsed since these crystalline albumins became available for study cannot be said however to have defined the

conditions necessary for obtaining a product with a just claim to actual chemical individuality.

It is quite certain that more than one proteid in egg-white remains unprecipitated after half-saturation with ammonium sulphate; and it is at least highly probable that on continued evaporation of the ammonium-sulphate-albumin mixture, in accordance with the original description of Hofmeister's process, more than one crystalline albumin eventually separates.

The well-known research of Bondzynski & Zoja published in 1894 offered strong grounds for believing this to be the case. . . .

Professor Hofmeister, it is true, has not himself observed the occurrence in egg-white of crystallisable albumins of varying solubility. . . .

I venture to think that there are grave doubts if the chief crystallisable albumin of egg-white has a sulphur content so low as that found by Hofmeister (cf. *infra*). . . .

There remains therefore, as I have said, some considerable doubt as to the real chemical individuality of the egg-albumin hitherto employed for various lines of study; for when, as in several recent researches, crystalline products have formed the material employed, no guarantee of purity has been sought other than that of the crystallisation itself. . . .

I shall now describe an application of the acetic acid method which I believe enables one to obtain quite easily a large yield of a well-characterised pure albumin . . . a procedure which is extraordinarily easy and rapid . . . by means of which a crystalline albumin may be separated from egg white, which upon repeated fractional crystallisation shows absolute constancy of rotary power and a constant proportion of sulphur.

This, it may be noted, is the first paper from Cambridge, and at much the same time a communication is made to the Cambridge Philosophical Society<sup>22</sup> on the same subject ("On the Separation of a Pure Albumin from Egg White," by F. Gowland Hopkins, M.A., M.B., B.Sc., University Lecturer in Physiology, from the Physiological Laboratory, Cambridge).

### *Tryptophane*

There follows next the celebrated work with S. W. Cole leading to the isolation of tryptophane.<sup>23,34,25</sup> This forms the subject of a series of three papers, proceeding in beautifully logical sequence. The first paper shows that the Adamkiewicz "acetic acid" reaction for proteins depends on the presence of an impurity, which is traced to glyoxylic acid. In the second paper the substance which is responsible for the reaction is isolated, and is shown to be identical with the hypothetical "tryptophane" of earlier workers. In the third paper the constitution of tryptophane is established, and its decomposition by bacteria studied.

The following extracts are taken from the first of this series of three papers ("by F. Gowland Hopkins, M.A., M.B., University Lecturer in Chemical Physiology, and S. W. Cole, B.A., Trinity College. From the Physiological Laboratory, Cambridge. Communicated by Dr. Langley, F.R.S.").

In 1874 Adamkiewicz described the now familiar reaction which results in the production of a violet colour when strong sulphuric acid is added to the solution of a proteid in glacial acetic acid.

In what follows it will be shown that the mechanism of the reaction has been wholly misunderstood. Proof will be given that the use of acetic acid introduces an extraneous and perfectly specific factor into the reaction, involving the addition of a substance quite necessary to the formation of the coloured product. This substance, moreover, is not acetic acid but an impurity, which, though very generally present, is admixed in varying quantity, and is occasionally absent.

We were led to pursue the following investigation by observing that, with a specimen of acetic acid in use in this laboratory last year, it was impossible under any circumstances to obtain the Adamkiewicz reaction. [It is certainly rare to find a specimen of acetic acid which yields no reaction, though many contain too little glyoxylic acid to give a satisfactory colour.]

After different specimens of acetic acid had been compared, and various possible impurities tested, the authors noted that the chromogenic substance could be distilled off. They proceed:

This result—the explanation of which becomes clear in the sequel—appeared to limit somewhat the ground we had to traverse in our search. . . . With such indications as these facts afforded, we now fortunately elected to experiment with various two-carbon compounds of typical structure, such as might conceivably arise from acetic acid, by oxidation or otherwise. . . . Pure glycollic acid was obtained from Merck. It gave no trace of a colour reaction with proteid solutions and sulphuric acid. The product of its oxidation by Fenton's method, reacted, however, in a perfectly typical manner, and Fenton & Jones have found that this product is glyoxylic acid. . . .

*Summary.*—The proteid reaction described by Adamkiewicz is not a furfurol reaction, but depends upon the presence of small quantities of an impurity in the acetic acid employed. . . . The substance essential to the reaction is glyoxylic acid.

Reading the second paper, one still feels one is sharing in a romantic adventure of science and not merely witnessing a discovery of technical interest for protein chemistry. The paper<sup>24</sup> is headed "A Contribution to the Chemistry of Proteids. Part I, a Preliminary Study of a Hitherto

# Undescribed Product of Tryptic Digestion." It opens as follows:

In a recent paper we showed that the colour reaction of proteids described by Adamkiewicz is obtained only when glyoxylic acid is present in the acetic acid employed. From the observations described in the paper referred to it follows that the Adamkiewicz reaction is in no sense a furfural reaction, and therefore that there is no evidence for the current conception of its dependence upon carbohydrate groups in the proteid molecule.

It becomes of interest therefore to know the nature of the molecular group actually responsible for the colour reaction. . . .

We endeavoured therefore to separate such a substance from the products of proteolysis, and have finally succeeded in preparing a beautiful crystalline product which gives the glyoxylic reaction in a typical manner.

For reasons which will be understood in the sequel the substance is to be obtained in greatest amount from the products of pancreatic digestion and may be separated with especial ease when casein is the proteid employed. . . .

Even the account of a precipitation is inverted with interest:

The use of mercuric salts as precipitants, though common, has been mainly confined to the chloride and acetate. The sulphate, however, enters into some special relations and throws down certain substances not generally precipitated by the salts more commonly employed. So far as we are aware no other sulphate has been but little used for the purpose of chemical separation, at any rate under the conditions in which we have employed it, namely, of a considerable proportion of free acid present. When about 5 per cent. of free acid is present it becomes a highly effective precipitant. . . .

After the treatment described the product is obtained as the residue from white glistening plates of highly characteristic appearance. . . .

The substance gives also the tryptophane reaction. It may be cautiously added to aqueous solutions of the compound, even being avoided, a fine red-rose colour is produced, and at the same time, when shaken with amyl alcohol the coloured product of the reaction is quantitatively taken up by the latter solvent, it being in this case the red-rose colour absorption which is seen where the "tryptophane reaction" has been obtained from the original extract of tryptophane. The tryptophane reaction so far as the chemical character of the reaction is associated with the compound we are dealing with is therefore a characteristic of the latter from the original digestion mixture. There can indeed be no question that our substance is the *body* of the red product of this familiar reaction. There is indeed no doubt that our substance is the *body* of the tryptophane reaction.

The formula corresponds rather to a skatol-amido-acetic acid. For confirmation of this conclusion a more careful further investigation.

The final paper in the trio gives the decision:

The constitution of tryptophane is skatol-amido-acetic acid.\* . . .

With the possible exception of tyrosine there is—when casein is employed—no product of tryptic digestion more easily obtained in the pure condition than tryptophane. Difficulties only arise in connexion with improving the yield. Unless certain precautions are attended to the first crop of crystal leaves syrupy refractory mother-liquors which refuse to give further crystals. . . .

This paper is noteworthy as being one of the first to employ bacterial decomposition as an aid to establishing chemical constitution.

*Action of bacteria upon tryptophane.*—The preliminary study of tryptophane having indicated so clearly that it is a skatol or indol derivative, we proceeded as a first step towards a fuller investigation of the substance to ascertain if it would prove to be the parent of those various compounds of the indol group, which, since the work of E. and H. Salkowski, Nencki, and Brieger, have been so well known as putrefactive products.

It has been long known that tryptophane arises at an early period during the putrefaction of proteids. Claude Bernard was the first to notice the fact. . . .

### *Miscellaneous Papers on Proteins*

Several further contributions have to be noted here in the field of protein chemistry, although rather out of order in any strictly chronological survey. These include a careful chemical and metabolic study of Bence-Jones protein, published in 1911,<sup>32</sup> a note on the protein of the vesicular fluid of the hedgehog,<sup>33</sup> and some later observations on the denaturation of proteins by urea.<sup>82</sup>

Turning over the leaves of the "Bence-Jones" paper (1911) we notice that the senior author now has the titles of M.B., D.Sc., F.R.S., and is described as Praelector in Biochemistry and Fellow of Trinity College; University Reader in Chemical Physiology. We read<sup>32</sup>:

The condition known as Bence-Jones proteinuria should not fail to interest the physiologist as well as the pathologist. The appearance, in the course of a disordered metabolism, of protein material which escapes breakdown, and passes the kidneys in such amount that the nitrogen contained in it may amount to a third or more of the total nitrogen excreted; the peculiar characters of the protein, and the fact that, though a highly complex substance forming colloid solutions, it may be passed in large quantities and for long periods by kidneys which remain impervious to plasma proteins, and which are—to all appearance at least—histologically

\* This view had to be revised after the synthesis of tryptophane, by Ellinger & Flamand, in 1907, by which it was proved to be, in fact, not a *skatol* but an *indol* derivative.



HOPKINS AND COLE WITH THE FIRST SPECIMEN OF TRYPTOPHANE, 1901.

"We endeavoured therefore to separate such a substance from the products of proteolysis, and have finally succeeded in preparing a beautifully crystalline product which gives the glyoxylic reaction in a typical manner" (p. 55).



intact: there are aspects of the condition of significance for all concerned with the processes of metabolism and excretion. A proper understanding of the disturbances involved could hardly fail to throw light on normal protein metabolism. The physico-chemical properties of the excreted product deserve, too, the attention of all concerned with the study of colloid solution. . . .

The present paper deals, more fully than any previously published work, with three aspects of the subject: the physical chemistry of the protein, particularly as regards the influence of electrolytes in determining its peculiar behaviour on heating; the pure chemistry of the substance, and especially its content of various amino-acids; lastly the metabolism of individuals excreting it, mainly on the lines of a comparison between the amount of protein and of the total nitrogen excreted.

Our observations have been made upon the urine of three persons, a woman and two men. The general properties of the protein were studied with material from all three cases. A complete analysis of it was made in two; so that a comparison became possible. The metabolism was followed for a long period in the case of the woman; briefer observations being made in the other cases. . . .

A comparison of the amino-acid yield strongly suggests that the protein in two cases excreted was identical, and the general characters, elementary analysis, etc., of the protein of the third case also suggest its identity with that of the others.

The Bence-Jones protein contains all the amino-acids characteristic of a typical protein, and is itself characterised by containing a high proportion of the aromatic groupings. . . .

The chemical examination of "the glairy milky fluid expressed from the vesiculæ seminales" of the hedgehog forms the appendix<sup>33</sup> to a paper by F. H. A. Marshall:

This crystalloid insoluble substance certainly formed a large proportion of the total protein of the vesicular fluid, and the separation of such a protein in quantity from a physiological secretion *in situ* seems to be a somewhat remarkable and exceptional phenomenon.

In 1922 a patent<sup>61</sup> for a method for preparing lactalbumin was taken out by Adeane, Whetham, Hopkins & Stewart.

Of more recent date is the publication of some observations on the denaturation of proteins by urea<sup>62</sup>:

Denaturation, though a phenomenon familiar objectively to all who handle proteins, involves a change of state of which the precise nature is yet obscure. The term itself is scarcely capable of adequate definition. It is only certain that native proteins dispersed in water as lyophil colloids suffer, as the result of diverse alterations in their environment, a change which is accompanied by complete loss of solubility in pure water or dilute salt solutions. . . .

An influence seemingly quite different from those already mentioned, but resulting in typical denaturation, is exerted by urea (and, as will be immediately indicated, by other related substances) when added in high concentration to native protein solutions. This phenomenon has received but little attention until recently. . . . The following preliminary and descriptive account of certain observations of my own (some were made so far back as 1899, though now extended) will suggest, I think, that the mechanism of this form of denaturation is worthy of close study.

#### IV. MUSCLE CHEMISTRY

We have now to return once more to our roughly chronological survey, and so to renew acquaintance with the classical paper of Fletcher & Hopkins published in 1907.<sup>28</sup> As previously mentioned (page 42), it was a happy circumstance which threw the two men together as colleagues in the Department of Physiology at Cambridge—Walter Morley Fletcher,\* already distinguished for his researches in the physiology of muscle, and Hopkins, expert biochemical analyst and biochemical philosopher. This contiguity not only resulted in their becoming devoted and life-long friends, but led to the singularly fruitful collaborative study of muscle chemistry: work which perhaps branched somewhat from the main stream of Hopkins's investigations.

If one were to try and compress into a nutshell the main conclusions reached, it would no doubt be somewhat to the following effect—that Fletcher & Hopkins examined the lactic acid produced by frog muscle under various conditions; and that they especially emphasised (and perhaps this is the most essential technical feature of the whole paper) that it is imperative to avoid any manipulative treatment, injury, or heating of the muscle—as all these give rise to a spontaneous production of lactic acid which will deceive the investigator. Their main result was that muscular exercise (“fatigue”) causes the formation of lactic acid but that the lactic acid disappears again during rest in the presence of oxygen—under *anaerobic* conditions, on the other hand, lactic acid accumulates in excised muscle. But so clipped a summary entirely fails to convey any impression of the magnitude of the original paper, a communication no less than 62 pages in length, having a general summary of results of about 2,500 words and a summary-within-a-summary of about 250 words. Our remedy will be to resort to the easier expedient of quotation.

Speaking of the difficulties in the estimation of lactic acid in muscle, and of the errors caused unsuspectingly by “irritation” of the muscle

\* Dr. (after Sir) Walter Morley Fletcher, F.R.S., Senior Tutor of Trinity College, Cambridge (1905–14), and later the first Secretary of the Medical Research Council (1914–33).

during the actual process of extracting the lactic acid from it, Fletcher & Hopkins write:

A study of this removal of acid can only be based upon a knowledge of the rate of survival of acid-production both in resting and in contracting muscle, under anaerobic and aerobic conditions; and before dealing with the question of oxidative removal it will be necessary in the first place to give an account of the main facts of this acid production. For it is notorious that, quite apart from the question of oxidative removal of lactic acid—which has not previously, we think, been examined—there is hardly any important fact concerning the lactic acid formation in muscle which, advanced by one observer, has not been contradicted by some other. Abundant lactic acid formation is said to accompany the process of natural rigor in a surviving muscle (du Bois Reymond, Ranke, Boehm, Osborne), but this is denied (Blome, Heffter); it is said to accompany contraction, and to mark the advance of fatigue (Heidenhain, Ranke, Werther, Marcuse), but this is also denied (Astaschewsky, Warren, Monarie, Heffter). Indeed, it may be said that since Ranke wrote in 1865, no description of the elementary facts of lactic acid formation in muscle, despite the fundamental importance of the subject, has been generally accepted.

We believe that the present confusion is not in chief, if at all, a result of the technical difficulties of lactic acid estimation, but that it is due to the difficulties inherent in the extractive treatment of an irritable muscle. For it is clear that in such a case no treatment for the extraction from muscle can be accepted which, acting itself as a stimulus, has among its effects an increase of the acid to be estimated. . . .

In view of the fallacies attending the use either of water or of alcohol for the extraction which must precede lactic acid estimation, we think, on a review of the literature, that it is not too much to say that we have as yet no trustworthy comparisons of the lactic acid content of resting and active muscle respectively, and that perhaps in no recorded observations has a genuinely resting muscle been available for examination.

The difficulty is overcome in the following manner:

We hope to show that in our experiments we have avoided these dangers of alcohol stimulation—the magnitude of whose effects we never suspected before trial—by using ice-cold alcohol, which has no appreciable stimulant action, while it retains its killing and coagulative influence. Immersion in alcohol has been followed by immediate rapid and thorough grinding (with sand) of the muscle ice-cold, in ice-cold alcohol. Of this and of the methods of experiment and estimation a detailed account will now be given. . . .

The “concluding remarks” summarise two of the main findings:

The proof obtained early in the course of this research that the lactic

acid content of muscle is profoundly affected by the nature of the treatment received before or during extraction, has enabled us, we believe, to explain some of the contradictions in the statements of others about the fundamental relations of acid production.

Our experiments leave no doubt that in the survival processes which precede the disappearance of irritability there is a steady increase not only of total acidity in the muscle but of lactic acid itself. Equally certain is it that in acid production during fatigue lactic acid takes a large and probably predominant share. The necessity for reinvestigating such fundamental questions as these, before proceeding to the closer study of the phenomena which was our intended task, has given this paper essentially the nature of a preliminary communication.

Ten years later, in 1917, Fletcher and Hopkins are chosen by the Royal Society to deliver the Croonian Lecture—a lecture which, appropriately enough, was instituted by its founder “in order to encourage the study of muscular motion.” It takes the form of a review of the subject as it then stood, “based largely upon researches carried out at the Physiological Laboratory, Cambridge.”<sup>47</sup>

A helpful summary and retrospect of the work of Fletcher and Hopkins may be found in the second of the Herter Lectures, given at Johns Hopkins University in 1921, and from it the following passages are taken<sup>61</sup>:

In 1898 W. M. Fletcher published an account of an observation which, like many other observations of great importance, was in a sense of simple nature. The technique which made it reliable was not simple, however, and the value of the observation arose from its quantitative nature. Properly estimated it will, in my opinion, be found to have wholly removed the foundation of a belief which, as we have seen, had long dominated animal physiology. . . .

A quantitative study was clearly necessary for further progress, and in 1907 it was my privilege to join forces with Fletcher in an attack upon the problem. Although the results of our somewhat laborious research have failed to affect the teaching of some textbooks we have the satisfaction of knowing that they have been the acknowledged point of departure for recent important studies.

We found that the confusion in the literature as to the quantitative relations of lactic acid in muscle were wholly due to faulty technique in dealing with the tissue itself. When the muscle is disintegrated as a preliminary to extraction for analytical purposes, the existing equilibrium is entirely upset. Interacting factors are brought into abnormal relations and the processes of change are greatly accelerated. A fresh muscle had been supposed to contain as much acid as one in rigor simply because

it had been produced in the former by the treatment which had preceded estimation. The biochemist had not sufficiently remembered the instability of his material. Fletcher and I, however, found it quite easy, by means of a simple method, not only to avoid starting the changes which led to the formation of lactic acid, but to arrest them at any point during their progress, and thus establish their time relations.

We were able to show that the accumulation of lactic acid in muscle occurs only in the conditions of anaerobiosis. With a proper oxygen supply it fails to accumulate at all. . . .

The next point established by Fletcher and myself is of fundamental importance. If a muscle which, by exposure to anaerobic conditions, has accumulated lactic acid, be placed in oxygen, the acid is removed. The occurrence of this removal under the influence of oxygen is significant in all that follows. It has been fully confirmed by the later work of Parnas and Meyerhof.

A recent commentator,\* in assessing the influence and importance of this work of Fletcher and Hopkins, has observed:

"Fletcher had been working on this subject since 1898 and had already begun the study of  $\text{CO}_2$  production in muscle on rational lines; when Hopkins joined in the work soon after coming to Cambridge a very fruitful collaboration began. . . . The great importance of this work was that it started the study of muscle fermentation and its relation to muscular contraction, a study which in the years that followed attracted many notable workers from all parts of the world, and developed into one of the most complete achievements of modern biochemistry, providing, even to-day, material for work of major interest and importance. It also stimulated important work on biological oxidations."

A brief note by Winfield & Hopkins published in 1915<sup>41</sup> dealt with "The Influence of Pancreatic Extract on Surviving Muscle." Their observations, as they explain, were based on the following considerations. The view had long been held that carbohydrate stored in the muscles serves as the precursor of the lactic acid which is formed there during the survival period; Embden and others having stated that the amount of this lactic acid is increased by addition of hexose-phosphate. As the pancreas is so intimately concerned in the metabolism of carbohydrate, Winfield & Hopkins thought it worth while to try the effect of adding pancreatic extracts to minced muscle. They found that the addition did, in fact, inhibit the formation of lactic acid.

\* M. Stephenson, *Biochem. Journ.*, 1948, 42, 161.

## V. DIET AND PROTEIN

A review, "The Utilisation of Proteids in the Animal," published in *Science Progress* for 1906-7, shows that the author's mind is now turning from the purely "biochemical" study of proteins (§ I-III) to that of their metabolic and nutritional functions. In the course of this review<sup>27</sup> he writes:

Some, impressed by the wide range of chemical capacities displayed by the body, may prefer to believe that such a substance as adrenaline may be prepared from the gland *de novo*, starting from indifferent nitrogenous precursors. . . . But I believe that to attribute such powers to an animal cell when simpler chemical possibilities are available is of the nature of a resort to vitalism, and is against what is so far known of metabolism. . . . [Indeed some] French workers have offered evidence, which is by no means conclusive, that tryptophane is a precursor of adrenaline. . . . However this may be, some experiments indicating that an individual amino-acid may be essential for special purposes in the body, other than tissue repair, will shortly be published from the Cambridge laboratories by Miss E. G. Willcock.

This last allusion is to the paper<sup>30</sup> on "The Importance of Individual Amino-acids in Metabolism," celebrated as one of the earliest contributions to the problem of the "specific biological values" of proteins; and from this paper we may make our next quotation. The summary runs as follows:

A dietary containing zein as its only nitrogenous constituent is unable to maintain growth in young mice. The addition of tryptophane (an amino-acid absent from the decomposition products of zein) to such a dietary does not make it capable of maintaining growth. On the other hand, this addition greatly prolongs the survival period of animals fed upon zein, and materially adds to the well-being of such animals. The addition of tyrosine (which is already present in zein), in equivalent amounts, has no such effect. It is suggested that the tryptophane is directly utilised as the normal precursor of some specific "hormone" or other substance essential to the processes of the body.

The following further passages convey an impression both of the scope of this paper and of its historical background:

A deficiency in a nitrogenous dietary need not necessarily be one of quantity; the form in which the nitrogen is supplied may determine its efficiency. Thus, in the familiar case of gelatine it is, of course, a qualitative deficiency which makes that substance unable to maintain nitrogenous equilibrium. It is generally supposed that this qualitative deficiency is occasioned by the absence from gelatine of certain molecular groups which

are present in true protein, but this hypothesis leaves unexplained the advantage possessed by gelatine over fats and carbohydrates as a protein sparer. It is assumed that gelatine, owing to its constitutional deficiencies, cannot repair tissue waste, but can replace protein in so far as the latter functions as a source of energy; sharing with the proteid some unexplained advantage over fats and carbohydrates in this latter capacity.

Recent advances in physiology seem to justify a fresh attack upon this subject, upon somewhat different lines. It now seems necessary to differentiate between the minimal amount of protein necessary for actual tissue repair and that required for total maintenance; we have no reason for assuming that they are the same. . . . The discovery of substances absolutely essential to life, highly specific, and elaborated in special organs, suggests that some part, at least, of the protein products set free in the gut may be used directly by these organs as precursors of such specific substances. In adrenaline, for instance, we have an aromatic substance absolutely essential for the maintenance of life, and it is probable that the suprarenal gland requires a constant supply of some one of the aromatic groups of the proteid molecule to serve as an indispensable basis for the elaboration of adrenaline. . . .

Previous work on these lines has had the study of nitrogenous equilibrium as its basis. Voit and Munk showed in their classical work that gelatine could not maintain such equilibrium, while Lüscher showed that the addition of tyrosine at least improved its powers in this respect. During the progress of the present research a paper has been published by Kaufmann giving the results of gelatine feeding with the addition of the missing amino-acids tyrosine and tryptophane. On replacing one-third of the proteid nitrogen by a diet of gelatine nitrogen equilibrium was no longer maintained. If now one-third to one-half of the gelatine nitrogen was replaced by nitrogen in tyrosine and tryptophane the animal could be—for a time at least—maintained in nitrogen equilibrium. . . .

*Methods.*—The protein-like substance “zein” first described by Gorham and later studied among others by Chittenden and Osborne, and especially by T. B. Osborne himself, is, as is well known, obtained from maize (*Zea mays*). It gives most of the proteid reactions—for instance Millon’s reaction and the xanthoproteic and biuret reactions. Its decomposition products have not been fully studied, but it yields leucine, tyrosine, and abundant glutamic acid. On the other hand, Kossel & Kutscher have demonstrated that lysine cannot be obtained from it, and, as Osborne & Harris have shown, it fails to yield the Adamkiewicz reaction (glyoxylic reaction), thus indicating the absence of the tryptophane group.

The whole significance of the observations to be described in the following section depends upon this absence of the indol group. . . .

A few years later the problem of “individual amino-acids” forms the subject of another paper (with Harold Ackroyd)<sup>44</sup>—incidentally of

interest as the first of the series to appear in the relatively new *Biochemical Journal*. It deals with the rôles of arginine and histidine:

We began our experiments in the belief that the molecules of arginine and histidine might prove to be used conjointly in metabolism as precursors of the purine ring. Our observations upon body weight, and, no less, others upon allantoin excretion, seem to show clearly however that either one of these two diamino-acids can with a considerable degree of efficiency subserve the functions of the other. When both are removed from the diet the nutritive failure and the fall in allantoin excretion are unmistakable. When either alone is restored in sufficient amount there is maintenance and even growth, while no marked fall in allantoin is observed. . . . It is suggested that this is because each of them can, in metabolism, be converted into the other. . . .

It is suggested that arginine and histidine play a special part in purine metabolism, probably constituting the raw material . . . for the synthesis of the purine ring.

## VI. DIET AND ACCESSORY FACTORS

From "qualitative differences in proteins" it is almost a natural step to other "minimal qualitative factors of the diet." The first indications of the approaching revolution in dietetics are to be found in an address given to the Society of Public Analysts in November, 1906, on "The Analyst and the Medical Man."<sup>\*26</sup> This paper deals primarily with certain difficulties which had arisen in the professional relationship between public analysts and medical officers of health, but it goes on to make some extremely significant statements about coming developments:

While upon the business of prophecy, I am tempted to put another series of prognostications before you, the credibility of which is at the present time, perhaps, more obvious to the physiological chemist than to anybody else. I pass from pathology to an aspect of dietetics. This is a subject in which the medical man is the recognised authority, charged with instruction of the public, but for a scientific knowledge of which he depends largely on the chemical physiologist and the analyst. . . .

Physiological chemistry, chiefly owing to the work of Emil Fischer, has recently gained the knowledge that individual proteins, and among them those which contribute to human dietaries, may each bear a special chemical stamp; that a given protein may differ so widely from another protein as to have, quite possibly, a different nutritive value. I will illustrate this, first of all, by a somewhat extreme case. A protein, zein, forming no inconsiderable proportion of the total nitrogenous constituents of maize,

\* Reproduced in full below, p. 123.

is entirely deficient in at least one characteristic molecular grouping. It yields on digestion no tryptophane, the product which represents the indol group present in the molecule of most typical proteins.

Recently we have fed animals with this indol-free maize protein in such a way that it formed the only supply of protein, though associated with abundant fat and carbohydrate and suitable salts. The diet wholly failed to maintain tissue growth in young animals, which, however, grew at once when their zein was replaced by pure casein. When tryptophane was added to the zein diet, there was still inability to maintain tissue growth, doubtless because the zein has other deficiencies as a protein. . . . In an extreme case a particular protein may wholly fail to support life, just as is the case with gelatin. . . . Here, there, or elsewhere in the organs must appear special, indispensable, active substances which the tissues can only make from special precursors in the diet.

The indol grouping in the protein molecule serves some such special purpose, quite distinct from its necessary function in tissue repair. This matter of qualitative differences in proteins may be of no small significance in dietaries. It may account for what I believe is proved by experience—that rice may serve the races which rely upon it as an almost exclusive source of protein, while wheat is only suitable for races that take a much more varied dietary. It may explain many variations in nutritive values which at present we feel and recognise only vaguely. In the future the analyst will be asked to do more than determine the total protein of a food-stuff; he must essay the more difficult task of a discriminative analysis.

But, further, no animal can live upon a mixture of pure protein, fat, and carbohydrate, and even when the necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or the tissues of other animals, and these contain countless substances other than the proteins, carbohydrates, and fats.

Physiological evolution, I believe, has made some of these well-nigh as essential as are the basal constituents of diet. Lecithin, for instance, has been repeatedly shown to have a marked influence upon nutrition, and this just happens to be something already familiar, and a substance that happens to have been tried. The field is almost unexplored; only is it certain that there are many minor factors in all diets of which the body takes account.

In diseases such as rickets, and particularly in scurvy, we have had for long years knowledge of a dietetic factor; but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure. They are, however, certainly of the kind which comprises these minimal qualitative factors that I am considering.

Scurvy and rickets are conditions so severe that they force themselves upon our attention; but many other nutritive errors affect the health of individuals to a degree most important to themselves, and some of them depend upon unsuspected dietetic factors.

I can do no more than hint at these matters, but I can assert that later developments of the science of dietetics will deal with factors highly complex and at present unknown.

In 1912 was published in the *Journal of Physiology* the paper<sup>34</sup> which drew wide attention to the theme of accessory factors and earned for its author the title of "der geistige Vater der Vitaminlehre." Its opening sentences convey the general scope of the investigation.

The experiments described in this paper confirm the work of others in showing that animals cannot grow when fed upon so-called "synthetic" dietaries consisting of mixtures of pure proteins, fats, carbohydrates and salts. But they show further that a substance or substances present in normal foodstuffs (e.g. milk) can, when added to the dietary in astonishingly small amount, secure the utilisation for growth of the protein and energy contained in such artificial mixtures.

The particular experiments, of which an account is now to be given, were undertaken to put upon a more quantitative basis results which I obtained as far back as 1906-1907. Since that time, a fuller realisation of the fact that (leaving on one side the influence of the inorganic constituents of dietaries) protein supply and energy supply do not alone secure normal nutrition, has arisen from the extremely interesting recent work upon the etiology of such diseases as beriberi and scurvy. (For references see Casimir Funk, this *Journal*, 1911, 43, 395; also *Journal of State Medicine*, 1912; and Holst, *Journal of Hygiene*, 1907, 7, 619.) It is not surprising that much work is now being done in connexion with the subject; and since the experimental results given in this paper were obtained, the publications of others have covered part of the ground. In particular I may refer to the work of Stepp (*Biochem. Zeitschr.*, 1909, 22, 452, and *Zeitschr. Biol.*, 1911, 57, 195) upon mice, and to the extensive researches of Osborne & Mendel upon rats.

In his Chandler Medal Address, given at Columbia University in New York, in 1922,<sup>62</sup> Hopkins gives the following personal reminiscences of this work.

During 1906 I was engaged in feeding animals upon mixtures of different amino-acids, and of necessity had to employ synthetic foods. Starch, fats and salts were added to the amino-acids to complete the dietary. . . . When, earlier, I began experiments with synthetic diets I added small quantities of meat extracts and extracts from yeast to give flavour to the tasteless food. I was thinking then of the animal's "appetite," as was natural, and as most people do when they start such experiments. I quickly found, however, that rats ate synthetic diets very well without such additions, and at the time of the experience with casein just mentioned I had ceased to use them. . . . Growth was now as good, and sometimes better than with the old casein, and growth occurred, too, in animals which were eating no more,

or even less, than other individuals living on the pure casein diet without addition and showing continuous loss of weight as a result. The extracts powerfully affected nutrition. . . .

By this time I had come to the conclusion that there must be something in normal foods which was not represented in a synthetic diet made up of pure protein, pure carbohydrate, fats and salts; and something the nature of which was unknown. Yet at first it seemed so unlikely! So much careful scientific work upon nutrition had been carried on for half a century and more—how could fundamentals have been missed? But, after a time, one said to oneself, "Why not?" The results of all the classical experiments had been *expressed* in terms of the known fundamental foodstuffs: but these had never been administered *pure!* If, moreover, the unknown, although clearly of great importance, must be present in very small amounts—again, why not? Almost infinitesimal amounts of material may have a profound effect upon the body, as pharmacology and the facts concerning immunity assure us. Why not then in nutritional phenomena? The animal depends ultimately upon the plant for the synthesis of materials which bulk largely in its food: there is no reason why it should not be adjusted so as to be in equal need of substances which the plant makes in small amount. Only if energy were the sole criterion of an animal's needs would this be impossible; but certainly it is not the sole criterion. . . .

When, therefore, at last, I ventured on publication it was mainly my experiments with milk that I described in proof of the existence of accessory food factors. . . .

I have given you a brief account of my own early experiences in this domain (never having told about them before) because I was assured that you would care to hear it. I fear you may have found it trivial, though the period it deals with contained some thrilling moments for me.

Belief in "vitamines" was not to win universal acceptance at first, and in the next year or two we find several papers of a polemical character. In 1913 Hopkins & Neville<sup>37</sup> have to write:

In a recent paper, Osborne & Mendel have described certain experiments which seem to show that young animals (rats) can grow when fed upon artificial diets consisting of "purified" constituents alone. . . .

The purpose of the present note is to indicate that there is still reason for a continuance of the search for special accessory substances of potent influence upon growth. It should be pointed out that Osborne & Mendel themselves admit that such substances may exist.

The vitamin value of milk presents an experimental difficulty<sup>55</sup>:

I may be allowed the privilege of saying here that the experiments which I described in 1912 followed upon a long experience . . . during which startling successes were mingled with puzzling failures. . . . In the

synthetic diet employed by me, the protein and the carbohydrate were purified to the uttermost, but I used little or no discrimination with regard to fats.

With milk, as an addendum to the purified foodstuffs, I got consistent results in all cases. . . . I am endeavouring to obtain further light on the matter. The purpose of this note is merely to point out that given the right conditions my original observations can be repeated.

Mention of two later contributions on vitamins, the first dealing with vitamin A and the second with vitamin C, must be included here. That on vitamin A (1920) refers to its stability to heat. Other workers had obtained somewhat contradictory results, and this paper (together with several others published almost simultaneously from other laboratories) clears up the puzzle by showing that the destructive influence is not heat *per se* but aeration<sup>56</sup>:

A knowledge of the conditions which affect the stability of vitamins is not only of immediate practical importance in connexion with the commercial and domestic treatment of foods, but is, clearly, not without value in setting certain limitations to hypotheses which may be framed as to the nature of these substances, and also in giving guidance when attempts are made to isolate them.

There is at the moment less certain knowledge of this kind in the case of the "fat-soluble A" than in those of the two other recognised vitamins. Thus Steenbock, Boutwell & Kent [1918] came to the conclusion that the substance is readily destroyed by heat, and, later, Drummond [1919], from the results of his earlier experiments, came to the same conclusion. Osborne & Mendel [1920], on the other hand, have recently confirmed earlier results of their own which indicated that it is resistant to heat. The investigation to be described in the present paper—to the results of which public reference has already been made [Hopkins, 1920]—confirms Osborne & Mendel's work by showing that the vitamin is relatively resistant to heat. It has demonstrated, on the other hand, that this nutritive factor is rapidly destroyed by exposure to atmospheric oxygen at temperatures ranging from 15° to 120° . . . .

Experiments carried out upon a large number of rats have shown that the fat-soluble A substance in butter, while displaying marked resistance to heat alone at temperatures up to 120°, is readily destroyed by simultaneous aeration of the fat, presumably because it is a substance prone to oxidation by atmospheric oxygen.

The work<sup>102</sup> on vitamin C, which was of much later date, was prompted by the desire to learn something about the process by which the rat is able to synthesise this vitamin, and about the nature of the precursors of the vitamin. A diet rich in carbohydrate, it was found,

caused the appearance of a localised concentration of ascorbic acid in the wall of the intestine, while in the absence of carbohydrate more was found in the liver.

The circumstance that certain animals, of which the rat is a typical example, synthesise ascorbic acid in the course of their metabolism raises questions of some scientific interest concerning the nature of its precursors and the seat or seats of its formation in the body. Experience with synthetic diets indicates that its precursors are derived from the main foodstuffs rather than from any minor constituent of the diet, and its constitution suggests, of course, that there would arise more directly from carbohydrate and perhaps also, less directly, from protein. As a preliminary to any attempt to determine the nature of possible intermediate products it seemed worth while to seek further information concerning its primary origin in the diet.

*Vitamin history in retrospect.*—To-day one is apt to forget the fact that for some time the "theory" of vitamins was hotly contested. In 1920 the British Medical Association held its annual meeting at Cambridge. A discussion took place on "The Present Position of Vitamins in Clinical Medicine," opened by F. Gowland Hopkins, Professor of Biochemistry in the University of Cambridge. Other contributors were Miss Harriette Chick, R. McCarrison, Professor A. F. Hess (New York), George F. Still, Leonard Williams, H. Cory Mann, W. H. Willcox, Eric Pritchard and S. Monckton Copeman. The opening speaker remarked<sup>32</sup>:

In what I am about to say I refuse to speak of the vitaminic "hypothesis." Vitamines, though still of unknown nature in the chemical sense, are not merely hypothetical. In nearly every case we are to consider, it is, I admit, still a hypothesis that the particular disease depends upon vitaminic deficiencies, but in respect to the broad aspect of nutrition as a whole, the importance of these factors is proven.

But I have found that there is at the present moment some scepticism concerning the whole question, particularly perhaps among certain members of the medical profession. The fact that I have met it lately in high quarters accounts for the particular direction I am giving to my opening remarks which may seem unnecessarily defensive.

Some of the scepticism has been stimulated by, or a protest against, quackery—the quackery which always dogs the footsteps of honest scientific work and sound views concerning human nutrition. It is well indeed that the subject we are to discuss should just now be approached critically; but disbelief in the very great importance of qualitative deficiencies in the diet (quantitatively of small moment) in the production of nutritional errors is to be deprecated.

Not long ago, in a professional discussion, a physician whom we all greatly respect and whose authority in such matters is of the highest, spoke of the "vitamine stunt" . . . the conception of vitamins is no stunt. It is based upon experiments as conclusive and as carefully controlled as any in biological science.

At this discussion "the opposition" was represented by Sir James Barr, M.D., LL.D., F.R.C.P., F.R.S.E., who said: "For anyone at the present day to deliberately say that he does not believe in 'vitamines' is tantamount to pronouncing his own anathema maranatha. Be that as it may, I feel that I must approach this subject in a sceptical frame of mind. . . . 'Vitamines,' so far as their composition is concerned, seem to be a figment of the imagination. . . . All these observations are easily explained without invoking any recondite influence of 'vitamines'." A different note was struck by Leonard Williams, M.D., of London: "It is very difficult to discuss with patience the views of those—so gently referred to by the opener—who hold that a deficiency does not give rise to disease. I am neither so patient nor so polite as Dr. Hopkins, and I have no hesitation in saying that such an attitude is both stupid and ignorant. Stupid because it displays a complete lack of imagination, and ignorant because it ignores ascertained and demonstrable facts."

A few years later (Cameron Prize Lectures, Edinburgh, 1923)<sup>68</sup> the enemy are almost in retreat:

When I recall certain published statements and certain oral expressions of opinion which have emanated from two important northern centres of scientific activity, I almost feel that I have crossed the border to accept a challenge!

But nothing is yet known about the composition of the vitamins:

I have some faith that we are near the moment when the constitution of one or another of them will be known. . . . The pursuit of pure chemistry has never involved analytical problems of such complexity, and there exists no classical technique for their solution. Maybe we shall have to replace the familiar technique of selective solvents and precipitants by some new technique.\*

Not a few lectures and prize addresses were given during these years, reviewing the growth of knowledge about vitamins: the "specific biological value of proteins," and allied topics in nutrition.<sup>43,48,53,57,62,103,104</sup>

With the coming of World War I the importance of nutrition in

\* In this address reference is made to some observations suggesting an imbalance between vitamins A and B.

the national economy gained official recognition: and soon a joint committee, appointed by the Medical Research Council and the Lister Institute, on "Accessory Food Factors" (of which Hopkins was the first chairman) issued a series of reports touching such matters, for example, as famine relief,<sup>30</sup> to be followed by a long series of technical memoranda<sup>31</sup> and monographs, including the several editions of the "Special Report on the Present State of Knowledge concerning Accessory Food Factors." The war years† saw also the publication of a booklet, "Food Economy in War-time,"<sup>32</sup> drawn up at Cambridge by the Drapers Professor of Agriculture and the Professor of Biochemistry, which bears the following introductory note.

It is well understood by the public that at the present time a rigorous economy in food is not only desirable on general grounds, but absolutely necessary to the success of this country in the task before it. While all may have the wish to economise, many will feel the need for some guidance with regard to the lines upon which economy may be practised without injury to health. Some such guidance this pamphlet is intended to supply.

Looking back on the "earlier history of vitamin research," on the occasion of the award of the Nobel Prize in 1929, Hopkins writes<sup>33</sup>:

The circumstances of my most enviable position here to-day will justify me in dealing rather with the earlier history of the subject, and I will venture in virtue of that position to put before you certain personal experiences which have no place in the proper history of the subject. They have not been, and will not be, published elsewhere. . . .

It is abundantly clear that before the last century closed there was already ample evidence available to show that the needs of nutrition could not be adequately defined in terms of calories, proteins and salts alone. How came it then that this limited definition was then in vogue and so remained for the first ten years of the present century, while no effective attention was given to the facts which have been discussed? . . . It is sure that, until the period 1911-12, the earlier suggestions in the literature pointing to the existence of vitamins lay buried. There is no evidence, I think, that they were affecting the orientation of any authoritative teaching concerning the phenomena of normal nutrition either at the time in question or indeed, in any effective sense, before. . . .

F. Rohmann, an experienced worker on nutritional problems, and much

† The paper by Ackroyd & Hopkins<sup>34</sup> referred to a few pages back contains the following introductory footnote: "Several of the experiments described in this paper were made in 1914 and the rest in 1915. My colleague has been long at the front, and in writing the paper I have been unable to consult him. He has had, moreover, no opportunity of reviewing the experimental results as a whole. If, therefore, it is held that the conclusions are not warranted by the facts, I alone am responsible." Ackroyd, in the Royal Army Medical Corps, gained the V.C., and was killed in action.

concerned with the chemical side of them, but one who never fully believed in the claims made for vitamins, wrote in 1916 after discussing the earlier literature, "Als der geistige Vater der Vitaminlehre ist wohl Gowland Hopkins zu betrachten, während die Bezeichnung Vitamine von Casimir Funk her stammt. . . ."

. . . Much correspondence received at the time from European colleagues made me feel then that my paper had served the purpose I had wished for it, namely, to direct thought concerning normal nutrition into a channel which, if not new, had been long and strangely neglected. For a time indeed I thought that channel to be even new. I was at least a pioneer whose efforts were not wasted, and I am always now content to recall an opinion expressed by the late Franz Hofmeister, the most just, if also the most generous of critics. Hofmeister, in 1918, after an exhaustive study of the whole literature speaks of me as the first to realise the full significance of the facts. If that be true, and if, as well may be, that has been the view of the Nobel Commissioners who have thought me worthy of so great a reward, I can happily enjoy my good fortune.

## VII. GLUTATHIONE AND TISSUE OXIDATIONS

The jump from vitamins to glutathione is explained by an introductory paragraph in the paper on "An Autoxidisable Constituent of the Cell."<sup>59</sup>

My attention was directed to the subject in two separate ways. Some years ago I was endeavouring to discover if vitamins were to be found among sulphur-containing compounds, and was led part of the way towards the separation of the substance now described. A little later, acting on the suggestion that acid formation in muscle is a necessary factor in contraction, I wished to discover if by chance, in the absence of carbohydrate, acetoacetic acid from fat might function instead of lactic acid. This led me to apply the nitroprusside test to tissues. At this time Arnold's papers had not yet appeared and I was ignorant of Heffter's publications. The above-mentioned inquiries were nugatory but they led to the present one.

The main outlines of the work is described in these terms:

The research described in this paper, though its actual point of departure had an intention quite different, ultimately resolved itself into an endeavour to throw light upon the chemical nature and physiological significance of a constituent of living tissues, which, though hitherto of unknown nature, has long earned a name. There can scarcely be a doubt that the substance to be described is the "Philothion" of de Ray-Pailhade.

In 1908 Heffter applied the nitroprusside test to a great number of tissues and tissue extracts and obtained positive results in many cases. . . . A little later Arnold found that a strong nitroprusside reaction may be given by

proteolytic extract of tissue, and came to the conclusion that free cystein was the responsible substance, . . .

Evidence is given to show that the substance is a dipeptide containing glutamic acid and cystein.

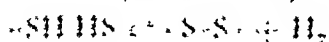
In the first paper on glutathione the question of its role in an oxidising system in animal tissue is already discussed:

Observations will now be briefly described to show that the dipeptide exerts a real function in the chemical dynamics of the cell.

After a consideration of current views on biological oxidations and reductions Warland's views on activation of hydrogen and Thunberg's on hydrogen acceptor - the text continues:

I have said thus much to indicate the importance which is now attached to the presence of acceptor, and especially of hydrogen acceptors, co-existing with substance to be oxidised. The dipeptide when in the disulphide (oxidised) form can of course under some conditions act as a hydrogen acceptor, and the labile hydrogen of the resulting sulphhydryl group, under other conditions, as an oxygen acceptor, . . .

Considerations may now be put forward which indicate that the dipeptide does as a matter of fact play a real part in cell dynamics -



The latter aspect is discussed further in a separate lengthy paper (Hopkins & Dixon, 1922).<sup>6</sup>

A full study of the general chemical properties of the substance is in progress. The present communication is concerned with observations meant to elucidate further its relations and functions in the living cell, and for the most part only with experimental results which have shown that its activities are associated in an unexpected manner, and to a remarkable extent, with tissue agencies which are thermostable.

The facts suggest that co-existing in living tissues with the specialised enzymic mechanisms is a thermostable mechanism for oxidations and reductions. Materials in some close association with structural elements are oxidised, anaerobically or aerobically, with the co-agency of the sulphur group in glutathione, . . .

Three years, indeed, before glutathione was isolated Meyerhof had fully considered the possibility that his *Atmungskörper* [respiratory substance] might be a substance carrying the -SH group. . . .

With regard to the substance glutathione it would seem that the properties of its sulphur group are such that once the presence of the dipeptide in a tissue has been shown, a recognition of the fact that it exerts a real influence in the processes of reduction and oxidation as they occur in that tissue follows almost as a corollary.

The -SH group under the physical and chemical conditions which exist in living tissues is certainly auto-oxidisable. It is equally certain that once the -SH group has given place to the -S-S- group as the result of such oxidations there are factors in the tissues which promptly restore equilibrium by fresh reduction of the latter.

These classical contributions on glutathione were followed up shortly afterwards by a paper by Morgan, Stewart & Hopkins,<sup>64</sup> the main point of which was to show that the well-known biological oxidation of purin bases to uric acid by xanthine oxidase, generally regarded as a process needing the presence of oxygen, could also be brought about under anaerobic conditions, provided methylene blue were present as a hydrogen donator.

Since the pioneer work of Burian, W. Jones and Schittenhelm, the conversion of the purin bases into uric acid by animal tissues has become a familiar case of biological oxidation. Apart from its physiological importance, the phenomenon must always be one of considerable chemical interest, because of the contrast between the remarkable ease with which it occurs under the influence of tissue catalysts, and the difficulty with which it is induced by ordinary laboratory oxidising agents. The literature of the subject seems throughout to emphasise the importance of free oxygen in the process. We have found, however, that active animal tissues can induce the oxidation under conditions which are strictly anaerobic. This fact is significant in its bearing upon the nature of the catalysts concerned.

Our inquiry began with the observation that in milk containing methylene blue, as a hydrogen acceptor, the bases are rapidly oxidised to uric acid in the absence of free oxygen.

Schardinger, in 1902, showed that milk, when quite fresh, does not reduce methylene blue, but reduces it when formaldehyde is also present.

A series of five further papers appeared within the next few years dealing with glutathione.\*

The problem of unravelling its mode of action, in promoting oxidations in the animal organism, presented some unexpected puzzles:<sup>70</sup>

This paper gives an account of observations which illustrate certain of the properties of glutathione as a promoter of oxidations. The fresh facts made available are as yet quite insufficient to explain its precise functions in living tissues, but they carry suggestions for future work. The experimental results touch at some points on phenomena concerning which basal

\* At about this time, Hopkins was also the author of a technical review, "Das Schwefel-System," which appeared in Oppenheimer's *Die Fermente und ihre Wirkungen*. This summarised some of the earlier literature, and gave working details of the techniques involved in the estimation of soluble thiol groups, the isolation of glutathione, and the anaerobic and aerobic methods for examining the influence of such sulphur groups on oxidation and reduction of tissues *in vitro*.<sup>71</sup>

chemical knowledge seems lacking. They cannot therefore be succinctly described.

Many of the observations described were made more than two years ago, but publication was unavoidably delayed. Unfortunately, more recent work—much limited by lack of leisure—still leaves the subject in a position which is curious and obscure. I think the fresh facts described are of real interest, however, and publication at the present time may stimulate the work of others. This paper should be looked upon, in part at least, as a prolegomenon to publications from this laboratory which will appear in the near future.

Measurements were made of oxygen uptake in Barcroft manometers, and it was shown that the oxidation of certain unsaturated fatty acids (or of lecithin) was accelerated by the presence of glutathione. Similarly, it promoted the oxidation of proteins containing an oxidisable -SH group.

Glutathione (GSH or  $G_2S_2$ ) promotes an oxidation of certain pure proteins on peculiar lines. The oxidation proceeds only when and while the protein displays (as shown by the nitroprusside reaction) an SH group. I am well aware indeed that on first acquaintance the curious, and at many points obscure, phenomena described in this paper may seem to lack biological reality. The experimental results described, however, are constant and easily reproducible. They are yielded by systems which contain actual cell constituents in contact with one another, and depend upon actual properties of these constituents. Such studies would seem to be a necessary, if remote, antecedent to a fuller understanding of the same constituents in the organised phenomena of the living cell. . . .

Meyerhof was the first to observe the oxidation of lipoids under the influence of a thiol compound. . . . Those who are familiar with Meyerhof's paper will remember that his own experimental studies finally led him to attach a predominant importance to the function of the SH group as an oxygen carrier (being especially active therefore in systems where its concentration is maintained) rather than to the circumstance that it is a reducing agent easily oxidisable to an S-S group, from which it is again easily reproduced by reduction. It was these properties which determined my own previously published views as to the importance of glutathione as a carrier of hydrogen. Nothing, I think, is now more sure than that *both* views *may* be correct.

It was in 1927 that the first suggestion was made (by Hunter & Eagle in America) that glutathione was not a dipeptide, as had at first been believed, but contained a third amino-acid in addition to glycine and cystein. Although this suggestion came as a surprise, and did not at first receive ready acceptance,<sup>74</sup> it led to an improvement in

the method of isolation,<sup>78,79</sup> and to other unexpected developments to be mentioned below.<sup>83</sup>

From the preliminary note in *Nature*<sup>78</sup>:

*A crystalline tripeptide from living cells.*—It has recently proved possible to isolate from cell extracts, for example, from extracts of yeast and red blood corpuscles, a tripeptide containing glycine, glutamic acid and cysteine, which readily crystallises. The separation is based upon the insolubility of the cuprous salt of the substance in normal sulphuric acid. This property of the salt makes cuprous copper an exceptionally selective precipitant.

The tripeptide is obtained in amounts which suggest that it is a cell constituent of importance. . . .

The isolation of this pure substance has indicated that "glutathione," as previously described by myself, is not an individualised substance. Preparations as described have contained a large proportion of the tripeptide. As a number of workers are employing such preparations in experimental work, it seems desirable that I should make the error known as soon as possible. A description of the tripeptide is in the press and will shortly appear in the *Journal of Biological Chemistry*.

The fuller report in the *Journal of Biological Chemistry*<sup>79</sup> gave details of the new and beautiful technique now introduced by Hopkins for separating pure crystalline glutathione:

The grave discomfort involved in making an admission of previous error is mitigated by the circumstance that I am now able to describe a method, not without special interest in itself, which with ease and rapidity separates from yeast and from red blood corpuscles a pure crystalline thiol compound with a peptide or quasipeptide structure. . . .

The difficulties of the position at this stage made it essential that a new method of isolation should, if possible, be worked out. I met success in this endeavour as a result of the following observation. . . .

The evidence that the crystalline substance, of which the isolation has been described, has the constitution of a tripeptide seems to be so conclusive that I have not hesitated to speak of it as such throughout this paper. The analytical data, the evidence of its equivalent weight, the results of titrating its amino and carboxyl groups, and the yield of the three amino-acids upon complete hydrolysis, all unite in support of this constitution.

Anyone who undertakes its isolation will find that the method described is extremely easy to carry out. They may feel, I think, that the unusually high selectivity displayed by its cuprous copper as an agent for separation has an interest of its own; and they will certainly realise that the tripeptide is to be obtained crystalline with an ease seldom found in dealing with compounds of its class. . . .

The present paper, with full intention, has followed lines that are merely descriptive. It is certain that conclusive views concerning the constitution

of the newly isolated compound must await the result of synthetic studies, and these, owing to the apparent difficulties experienced in the preparation of cysteinyl derivatives, may prove troublesome.

The isolation of glutathione in the purified crystalline form was followed by the surprising discovery<sup>88</sup> that, unlike the original crude substance, it failed to stimulate oxygen uptake when added to washed tissues:

It has been commonly assumed that one function of glutathione in the tissues (others have been suggested) is to act, in virtue of its sulphur groupings, as a carrier of hydrogen from reducing systems to molecular oxygen. This assumption has hitherto been based upon its behaviour when added to washed tissue preparations.

The observations of Meldrum & Dixon (1930), however, have shown that the tripeptide, after its separation from the tissues in crystalline form, while freely reduced by tissue preparations, undergoes oxidation by molecular oxygen only with the co-operation of two factors present as traces in most preparations of the substance; not alone iron (or copper) of which the necessity was long ago shown by Warburg and Harrison, but also some substance able to form catalytically active complexes with the metal. Unlike earlier, less pure, preparations the crystalline product when added to washed tissues entirely fails to produce a system capable of taking up oxygen. . . .

The demonstration of this stability of the tripeptide towards oxygen after isolation made it desirable that its fate when present in fresh intact tissues should be followed during the course of survival respiration. Its behaviour while thus retaining its normal associations in the cell should then be displayed.

The results to be described, in so far as they refer to its oxidation, have been obtained chiefly from studies of liver tissue. The reduced (thiol) form of glutathione as it exists in the liver we have found to be readily oxidised to the disulphide form by molecular oxygen. We have also found that its oxidation during the survival respiration of the fresh tissue is associated always with a continuance of reduction processes, showing that the transport of hydrogen to oxygen is thus maintained, and indicating that the oxidation of the tripeptide has real metabolic significance. . . .

The main purport of the paper, however, is to describe events during aerobic survival respiration.

What our experiments have actually demonstrated is that in the course of these events there is sustained oxidation and reduction, respectively, of thiol and disulphide groupings in the tissue. The evidence which suggests that glutathione is the dominant, and possibly the only, substance involved in these changes is sufficient to justify the title of this paper and the special reference to glutathione throughout.

In a later paper,<sup>112</sup> §§ VI and VII join hands again. The subject is a study of "some relations between ascorbic acid and glutathione," and it is shown that glutathione under suitable conditions performs the function of protecting the vitamin from oxidation. The introductory paragraph reads:

Ascorbic acid and glutathione are the most conspicuous and, so far as is at present known, the most active reducing substances in living tissues. In spite of the fact that their fundamental constitutions and physiological functions are so different, they have certain qualities in common. Both agree for instance in the circumstance that though their reduced and oxidised forms may co-exist in a tissue, they form redox systems which are not thermodynamically but only chemically reversible. Though in a given case the function of either may be specific, as is that of glutathione in the glyoxalase system, other systems are known in which one can replace the other, probably because in such cases reducing power alone determines their influence. Doubtless other more specific kinetic functions may be revealed in the future. Meanwhile the question arises whether, as reducing substances with different redox potentials, they can exert combined activities, or display interrelations of importance. This paper deals with their mutual relations as displayed in particular circumstances.

The findings are summarised in the following terms:

When ascorbic acid and glutathione are together in the presence of the hexoxidase described by Szent-Györgyi, the glutathione wholly protects the vitamin from oxidation, whilst it is itself oxidized at a rate which, with the same concentration of enzyme, is exactly the same as the rate with which ascorbic acid is oxidised when alone. Only when GSH has practically disappeared from the system does the oxidation of ascorbic acid begin.

When ascorbic acid has been reversibly oxidised its reduction by pure glutathione alone is a very slow process; but in the presence of the enzyme (in conditions which are discussed in section I) the reduction may be five times as fast as the oxidation induced by the same concentration of the enzyme.

Glutathione also completely protects ascorbic acid from oxidation by copper catalysis. The mechanism of protection must here be different from that which operates in the case of the enzyme. In the latter it depends upon hydrogen transference, in the former on inhibition of the catalysis.

In the last section of the paper the behaviours of ascorbic acid and glutathione in aerated hepatic tissue are described and discussed.

Later, in 1938, a paper by E. M. Crook & Hopkins<sup>120</sup> mentions that "Kertesz has stated [1938] that he was unable to repeat these experimental results" of Hopkins & Morgan (1936)<sup>112</sup>:

It became clearly desirable that the experiments should be repeated, and the present paper deals with such repetitions together with some extensions. They have involved the use of many different enzyme preparations, and they show that the results published by Hopkins & Morgan are invariably reproducible. . . .

In this paper it is shown that all the results of Hopkins & Morgan in their study of the system under reference are reproducible, and we may add that when the experimental conditions are properly defined the reproduction has been in our experience invariably. In fact, to be used the same materials as those used by them, the possibility of failure to obtain the same results is exceedingly difficult to envisage.

The experiments of Hopkins & Morgan on the system ascorbic acid-glutathione reductase, the ascorbic acid-glutathione reductase, were apparently unable to confirm, have been repeated, and the original results completely confirmed. In particular, the effect of variations in pH on the enzymic activity of ascorbic acid-glutathione reductase has been determined.

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great a multitude of distinct catalytic agencies.”<sup>85</sup> But, for the next step:

I will ask you to consider whether catalysis on highly specific lines is not among the most fundamental and significant phenomena in nature, and whether as displayed amid the complexities of the cell it is not as truly an essential attribute of life as any other physical attribute whatever. . . .

I will now come, therefore, to what is perhaps the chief purpose of this lecture, by boldly making a particular claim. This claim, based upon a belief in the organising capacity inherent in highly specific catalysis, is that at a level of organisation, determined by its physical and chemical attributes alone, the cell already constitutes a system which can maintain itself as an individual entity in dynamic equilibrium with its environment, and so, to that extent at least, display one of the characteristic properties by which we recognise it is alive. Such a claim some minds will almost instinctively reject. I believe, however, that most of those engaged in studying living tissues by chemical methods implicitly believe in it or in something approaching it. It is not often, perhaps, stated quite so explicitly. It seems justifiable to me, not from any predilection for chemical descriptions, but because my thought concerning the wide potentialities inherent in highly specific catalysis has led me to believe that it is in no way fanciful.

This conception is further developed in another address<sup>95</sup>:

The organising potentialities inherent in highly specific catalysis have not, I believe, been adequately appraised in chemical thought. The concentration of a catalyst or, alternatively, the extent of its active surface will determine the velocity of changes due to its influence, but highly specific catalysts determine in addition just what particular materials, rather than any others, shall undergo change. In this respect they are like the living cell itself, for they select from their environment. . . . I do not expect that all will feel able to admit as much as I myself would like to claim, namely, that the control of events by intracellular enzymes, exerted in the specialised colloidal apparatus of the cell by itself, secures the status of the cell as a system which can maintain itself in dynamic equilibrium with its environment. I am not denying for a moment that the cell has esoteric qualities which may call for organising influence of a greatly different kind, exerted maybe at some higher level. It is at any rate sure that the interrelated activity of highly specific catalysts represents a notable device of Nature which has supported during the course of evolution those dynamic manifestations which characterise living things.

Or again<sup>93,100</sup>:

We must recognise, however, that life has one attribute that is fundamental. . . . The arrest of energy degradation in living Nature is indeed a primary biological concept. Related to it, and of equal importance, is the concept of organisation.

## VIII. THE ADVANCE OF BIOCHEMISTRY

This brings our series of quotations nearly to the end of Hopkins' active scientific life, but there remain still a few gaps to fill in. At this point we shall be vividly reminded of how completely the biochemical scene has been transformed in these past 40 or 50 years, with the growth of knowledge, the development of new ideas, and with a changed outlook.

If we turn up any of Hopkins' earlier papers, we are immediately struck by the fact that the literature cited is almost exclusively German. In 1900 the *Zeitschrift für physiologische Chemie* was already in its thirty-first volume; not until 1906 did the British *Biochemical Journal* come into existence.\* It is difficult to mention a single British biochemist as a contemporary of the great continental names of the closing decades of last century—Hofmeister, Kossel, Fischer, Hoppe-Seyler, Hammarsten, Salkowski. There were one or two who, it is true, might justly be termed "chemical physiologists" (with the emphasis strongly on the second word), such, for example, as Halliburton. In 1913 Hopkins, the first of the British biochemists in the full sense of the term, succeeded Halliburton as author of the "Annual Reviews on Physiological Chemistry" in the Chemical Society's *Annual Reports*. He continued these reviews for five successive years,<sup>38,39,40,45,46</sup> to be followed in turn by George Barger. In the first of his essay-contributions we read<sup>39</sup>:

Each successive year brings proof that biochemistry is now attracting workers in rapidly increasing numbers. The output of papers in 1913 was very large, over five hundred researches being described in the *Biochemische Zeitschrift* alone. It is doubtful if really new ground has been broken. A retrospect of the year must for the most part deal with the development of enterprises begun before it opened. No paper, I think, has appeared which to a reviewer gifted with no more than ordinary insight suggests the opening of a new chapter in the science. Progress, nevertheless, has been very real.

If one were to endeavour to decide upon what is the most hopeful feature of the moment, I think it would be found in the increasing provision of accurate quantitative methods applicable to the determination of metabolites upon present minute amounts. . . .

A year later the war atmosphere is apparent<sup>39</sup>:

\* The series of monographs on biochemical subjects, of which Hopkins and Plimmer were joint editors, began to appear in 1908; the promised volume on *The Development and Present Position of Biological Chemistry*, by the first-mentioned editor, unfortunately never appeared.

Although the source which usually supplies the bulk of papers dealing with biochemical subjects was cut off when the year had run but little more than half its course, I have not found much relief from the embarrassment with which a plethora of material overtakes the writer of an annual review . . . one really feels that most of those papers which, having read, one wishes to discuss, are just now written in the English language.

In the nineteen twenties biochemistry was still understaffed, and the need for expert recruits is stressed in many addresses, e.g.<sup>62</sup>:

The study of nutrition is most productive when it is followed as a branch of applied organic chemistry. . . . Chemistry is a basal science underlying the practice of so many human activities that a large proportion of those who start with a chemical training must ultimately add to their equipment other kinds of expert knowledge before qualifying for their life's work. It is a pity that so few up to now have chosen biological qualifications. Hitherto, the primary training of most of those who have investigated biochemical problems has been biological or medical. Such workers have done very well, but as knowledge progresses it becomes more and more necessary that at least some of the work should be done by those whose chemical knowledge is primary and not secondary. . . . I can say with certainty that, in Great Britain at any rate, there is a demand for professional biochemists which is greatly in excess of the present supply. This I find satisfactory, for if a profession opens up we shall find it easier to obtain workers who during one period at least of their career will help advance the science itself.

I find it difficult, when addressing an audience composed largely of chemists, to avoid a propagandist attitude: because it is so very desirable that a proportion of our young chemists, greater than heretofore, should devote themselves to biological problems. I will confess that I am at the moment thinking in particular of organic chemistry.

By 1934 so much progress has been made in this direction that it is possible for the President of the Royal Society to say in his "Anniversary Address" to that body<sup>97</sup>:

In a previous address I have voiced the satisfaction that all concerned with the chemistry of living things must feel in the help that is now forthcoming in full measure from distinguished organic chemists towards the elucidation of the molecular structure of such substances as vitamins and hormones, with their great biological importance.

The separate biochemical laboratory, as distinct from the Institute of Physiology, is in itself only a fairly recent development. In 1926, at the Stockholm Congress,<sup>72</sup> the Head of the Cambridge Biochemical

Laboratory deals with the "Institutional Needs of Biochemistry," the "Need for Specialised Institutes of General Biochemistry":

I am among those who believe that independent Institutes of Biochemistry with specialised staffs for teaching and research should in every university stand by the side of the existing Institutes of Physiology. . . .

The greatest need of biochemistry at the moment in my opinion is equipment which shall make possible the study under one roof (of course from its own special standpoint alone) of all living material.

But a watching brief must still be held if the interests of the biochemist are to receive their just consideration:

I seem to have sensed the beginning of a definite movement in this country, and indeed elsewhere, not, of course, to ignore the laboratory; but in the distribution of funds provided for medical research to endow the clinic on a scale which might endanger the future of research in fundamental biological science. The tenor of my remarks has been due to a conviction that in the long run such a policy would sterilise advance.

If one wished to impress upon the youthful student of biochemistry the progress made between the two world wars, it would form an instructive exercise to request him to read, and compare, two addresses given by Hopkins before the British Association, the first,<sup>56</sup> as Chairman of the Physiological Section, at Birmingham, in 1913,\* the second,<sup>57</sup> as President of the Association, at Leicester, in 1933.† In 1913 it is still necessary to stress the need for trained chemists in biology (already touched on above):

. . . One can, I think, honestly say that it is yet a rare thing in this country to meet a professed biologist, even among those unburdened either with years or traditions, who has taken the trouble so to equip himself in organic chemistry as to understand fully an important fact of metabolism stated in terms of structural formulae. . . . I feel justified in repeating to-day the appeal of Liebig to the leading chemists of this country, in the hope that they may see their way to direct the steps of more of their able students into the path of biochemistry. . . . I have been in a position to review the current demand of various institutions, home and colonial, for the services of trained biochemists, and can say, I think with authority, that the demand will rapidly prove to be in excess of the supply. . . .

I have made it my business during the last year or two to learn, by means of indirect and most diplomatic inquiries, the views held by a number of our leading organic chemists with respect to the claims of animal chemistry. I do not find any more the rather pitying patronage for an inferior discipline,

\* Reproduced in full below, p. 136.

† Reproduced in full below, p. 242.

and certainly not that actual antagonism, which fretted my own youth; but I do find still very widely spread a distrust of the present methods of the biochemist, a belief that much of the work done by him is amateurish and inexact. What is much more important, and what one should be much more concerned to deny (though but a very small modicum of truth is, or ever was, in the above indictment), is the view that such faults are due to something inherent in the subject. . . .

One reason which has led the organic chemist to avert his mind from the problems of biochemistry is the obsession that the really significant happenings in the animal body are concerned in the main with substances of such high molecular weight and consequent vagueness of molecular structure as to make their reactions impossible of study by his available and accurate methods. There remains, I find, pretty widely spread, the feeling—due to earlier biological teaching—that, apart from substances which are obviously excreta, all the simpler products which can be found in cells or tissues are as a class mere dejecta, already too remote from the fundamental biochemical events to have much significance. So far from this being the case, recent progress points in the clearest way to the fact that the molecules with which a most important and significant part of the chemical dynamics of living tissues is concerned, are of a comparatively simple character. . .

I am certain that the search for tissue products of simple constitution has important rewards awaiting it in the future, so long as physiologists are alive to the dynamical significance of all of them. Such work is laborious and calls for special instincts in the choice of analytical method, but, as I mentioned in an earlier part of this address, I am sure that high qualifications as an analyst should be part of the equipment of a biological chemist.

On ultimate analysis we can hardly speak at all of living matter in the cell; at any rate, we cannot, without gross misuse of terms, speak of the cell life as being associated with any one particular type of molecule. *Its life is the expression of a particular dynamic equilibrium which obtains in a polyphasic system.*

This address is outstanding as being one of the earliest to view biochemistry from this “metabolic” or “dynamic” angle (its title is “The Dynamic Side of Biochemistry”), and it made a correspondingly great impression on contemporary thought.

In the Presidential Address for 1933<sup>93</sup> the topics touched on include: the recent work on hormones and vitamins; the “specificity of biological catalysis”; “chemical organisation” in the cell—to mention but a few; the underlying theme being “the importance of molecular structure in determining the properties of living matter.”

The student will find many of these same themes touched on in Hopkins’ address, “Chemistry and Life,” the Gluckstein Memorial Lecture for 1932, delivered before the Institute of Chemistry in 1933.<sup>94</sup>

As time went on, the recognition of the public importance of biochemistry became so widespread that inevitably Hopkins became involved in governmental administrative activities. There was the report of the Government inquiry on the pasteurisation of milk (the "Hopkins Committee"); the work of the Medical Research Council, and many other official bodies; occasional "anniversary" addresses<sup>76,104</sup> or obituary notices,<sup>31,35,66,90,98,99,125,126</sup> contributions to numerous discussions,<sup>54,81,84</sup> inaugural orations, commemorative and public lectures<sup>62,69,91,101,103,107</sup>; and, last but not least, the series of five informative and stimulatingly speculative Annual Presidential Addresses to the Royal Society.<sup>89,95,97,106,108</sup>

Many of us would wish to learn the recipe by which leisure could be snatched, in the midst of such manifold distractions, for the accomplishment of fundamental research work of the kind which it has been our purpose to follow in the foregoing extracts.

Perhaps the secret is to be found in the words of the final paragraph of the Presidential Address to the British Association at Leicester<sup>93</sup>:

You may feel that throughout this address I have dwelt exclusively on the material benefits of science to the neglect of its cultural value. I would like to correct this in a single closing sentence. I believe that for those who cultivate it in a right and public spirit, science is one of the humanities; no less.

## IX. THE LAST YEARS (1937-47)

Hopkins' term of office as President of the Royal Society came to a close in 1936. He was then 75 years old. In 1937, to honour his 75th birthday, 31 "past and present members of his laboratory" each presented him with an essay, and these were published together in a volume called *Perspectives in Biochemistry*.<sup>\*</sup> At this time, in his writings and addresses, Hopkins frequently refers to his research career as being almost closed. Nevertheless, despite indifferent health and growing loss of sight, he remained working in his laboratory for another 10 years, almost to the very end of his life, in 1947. A characteristic of this period is that he returned, perhaps in reminiscent mood, one by one, to almost all the research interests of his earlier

<sup>\*</sup> "*Perspectives in Biochemistry: Thirty-one Essays Presented to Sir Frederick Gowland Hopkins by past and present members of his Laboratory.*" Edited by Joseph Needham and David E. Green, Cambridge, 1937. (The authors of the essays are N. K. Adam, E. Baldwin, D. J. Bell, J. D. Bernal, A. J. Clark, M. Dixon, E. Friedmann, D. E. Green, J. B. S. Haldane, L. J. Harris, R. Hill, Barbara Holmes and Antoinette Pirie, E. Holmes, H. A. Krebs, R. Lemberg, Dorothy J. Lloyd, J. M. Luck, I. S. Maclean, J. Marrack, E. Mellanby, J. Mellanby, R. Scott-Moncrieff, Dorothy M. Needham, J. Needham, R. A. Peters, N. W. Pirie, J. H. Quastel, H. Raistrick, Marjory Stephenson, A. von Szent-Györgyi, V. B. Wigglesworth.)

years—vitamins, nutrition, enzymes, and even (in 1941) to his very first love, the pigments of butterflies' wings. This reflective mood of his last period is admirably caught in the picture painted by Meredith Frampton for the Royal Society, which shows him seated at a laboratory bench, flanked by a Soxhlet extractor, holding in his hand a small spectroscope, and meditating on an entry which he has made on his writing-pad—a reference to his own paper of forty-five years earlier, in the *Philosophical Transactions* for 1896.

In some half-dozen papers between 1938 and 1945 he continues to deal with glutathione in relation to enzyme systems and tissue oxidation—problems which had been occupying so much of his attention during the inter-war years.

### *Glutathione and its Relation to Oxidative Reactions and Enzyme Systems*

Hopkins' investigations of the effect of glutathione on the oxidation of ascorbic acid, including the confirmatory note published in 1938,<sup>120</sup> have already been mentioned (p. 78). After this he undertook in turn studies of its rôle: (a) in the light-sensitive system *riboflavin* plus *ascorbic acid*, and (b) in the action of the enzyme systems *succinic dehydrogenase* and *glyoxalase*. Another paper related to the appearance of glutathione during the germination of seeds.

*Photocatalysis in the system ascorbic acid, riboflavin and glutathione.*—The starting-point of this research<sup>119</sup> was the observation of Kon & Watson that in milk exposed to light there is a rapid loss of ascorbic acid by oxidation. Hopkins was able to show that this oxidation was "very actively promoted by the presence of small amounts of lactoflavin in its solutions." A second finding was that glutathione, by reason of its reducing action, inhibited the oxidation:

Kon & Watson (1936), in a very thorough study, have shown that exposure to light induces the oxidation of vitamin C in milk. In aqueous solutions however at pH 7.4 pure metal-free ascorbic acid is not affected by solar radiations, whereas in the presence of even very minute concentrations of the flavin oxidation proceeds; while with greater, but still small, concentrations it becomes very rapid.

In the course of experiment moreover the circumstance was revealed that this activity is not lost when the flavin itself has been decomposed by light. Certain, at least, among the less coloured products of such decomposition are efficient as promoters of the oxidation. In particular the lumichrome of Karrer, the molecule of which lacks the ribityl side chain, was found to behave similarly to the original flavin.

It is a further circumstance of interest that, as in other cases of ascorbic

acid oxidation, the photocatalysed process is inhibited by the presence of glutathione in adequate concentration. . . .

While the activity of lactoflavin as a sensitiser in the oxidation of ascorbic acid by light is a special instance of a not uncommon phenomenon, the fact that both substrate and sensitiser are substances of biological importance, and both vitamins, adds to the interest of the case. . . .

Whether the phenomena described have any immediate biological significance is perhaps not sure. There can be little doubt that the presence of flavin contributes to the photochemical oxidation of ascorbic acid in milk studied by Kon and his colleagues. . . .

*Serumflavin.* Pure ascorbic acid in buffered aqueous solutions at pH 7.4 is wholly resistant to solar radiation, but in the presence of small concentrations of lactoflavin as a sensitiser it undergoes a rapid photocatalysed oxidation. Lumichrome, though less active than the original flavin, is also an efficient sensitiser.

The simultaneous presence of reduced glutathione in adequate concentration inhibits the oxidation. . . .

*Glutathione and succinic dehydrogenase.*—Three papers in 1938-39 discussed the influence of thiol groups—in the form of glutathione—on the activity of certain dehydrogenase enzymes. In the first of these papers,<sup>117</sup> with his research assistant, E. J. Morgan, it was found that the presence of the thiol group was necessary for the activity of succinic dehydrogenase, as it had already been shown to be by investigators elsewhere for certain other enzyme systems:

The work of Heffter (1907), in which the existence of labile thiol groups in tissues was demonstrated, and his enlightened prediction that they would be found to play a noteworthy part in tissue respiration, suffered neglect for several years. In the paper containing the first account of glutathione (Hopkins, 1921) it was pointed out that the presence of that substance in the oxidised no less than in the reduced form accelerated the reduction of methylene blue by partly washed tissues, and that this was because by its reduction and subsequent oxidation it acted as an intermediate transporter of hydrogen from tissue to dye. This seems to have given the first hint that intermediate H transport might be a process proper to living tissues. . . . These relations correspond in effect to an equilibrium reaction between heterologous thiols and disulphides.



Recently Bersin & Steudel (quoted Bersin, 1936) have studied such a reaction in the case of cystine and thiolacetic acid.

Grassman *et al.* (1929), having the above-mentioned facts in mind, showed that papain and kathepsins can be activated by cysteine or by reduced glutathione; since then many researches have dealt with the influence of

thiol compounds on the activity of hydrolysing enzymes. It has become clear, in many cases at least, that thiols can activate such enzymes, and this because of their reducing powers. . . . Finally, evidence has accumulated to suggest, or perhaps to prove, that in many cases it is the fixed -SH groups in the protein element of each enzyme which suffer these reversible processes of oxidation and reduction. "The active enzyme is thus to be looked upon as a thiol compound while its inactive form is a disulphide compound" (Bersin, 1936).

As this paper is concerned, not with hydrolytic enzymes, but with dehydrogenases, it does not seem necessary or justifiable to refer in detail to the now extensive literature dealing with the former which has been briefly reviewed above. Bersin, whose own work has contributed much to the subject, has more than once dealt fully and critically with the literature, and in his reviews full references are to be found (Bersin, 1935; 1936).

In the case of the dehydrogenases very little work seems to have been done in this particular field. Wagner-Jauregg & Möller (1935) studied, it is true, the activation of hexosephosphatase and alcohol dehydrogenase, and came to the conclusion that glutathione activates these enzymes by immobilising inhibitory metallic ions.

A preliminary study, now to be described, of the behaviour of dehydrogenases has shown that, in one at least, it is exceptionally easy to demonstrate the apparent importance of the protein -SH groups for the enzymic activity, and, no less, the influence of glutathione in maintaining the integrity of these groups.

This is the succinic dehydrogenase, with which, and with the glycerophosphate dehydrogenase, this paper is chiefly concerned. . . .

The experiments described show unequivocally in the case of the succinic dehydrogenase that when the enzyme is exposed to the oxidising influence of GSSG its activity disappears simultaneously with the disappearance of the tissue-thiol groups, while both reappear together under the reducing influence of GSH. . . .

It seems sure that the integrity of a thiol group (or groups) in its structure is essential for the activity of the succinic dehydrogenase. . . .

The experiments described in this paper have proved the unmistakable importance of thiol groups only in the case of the succinic enzyme. . . .

The authors go on to discuss whether the activation by GSH may be common to the other dehydrogenases:

But this is perhaps the most typical among dehydrogenases; it is widely distributed among animal tissues, and the succinate system seems to hold a prominent position in the chemical economy of living cells. If thiol groups play a leading part in the mechanism of its action their influence will yield an objective illustration of an aspect of specific structure in correlation with specific functions. . . .

The behaviour of certain other dehydrogenases was studied but requires further elucidation.

However, in the second paper of the series,<sup>121</sup> with E. J. Morgan & Cecilia Lutwak-Mann, a negative finding has to be reported:

The experiments described support the view that the succinic dehydrogenase requires for its activity the integrity of SH groups in its structure while this is not the case with other typical dehydrogenases. . . .

The third paper of the series<sup>123</sup> alludes to the relation between the enzyme and the cytochrome system:

It has long been held that the succinic dehydrogenase requires no co-enzyme or intermediary hydrogen carrier to link its activity with that of the cytochrome system.

Ahlgren, however, working with a preparation made by extracting washed horse muscle with a solution of disodium hydrogen phosphate, found that with added succinic acid it induced an oxygen uptake which, though vigorous at first, rapidly fell to near zero. In the presence, however, of a *Kochsäft* from rabbit-muscle, the uptake was greater and much longer sustained, indicating that a thermostable factor may be necessary to complete the activity of the enzyme. . . .

Preparations of the succinic dehydrogenase are described . . . which, while very active in reducing methylene blue, fail completely to induce an oxygen uptake in a system containing succinates, cytochrome *c* and cytochrome oxidase. It is clear, therefore, that some factor is necessary for transferring the hydrogen atoms activated by the enzymes to the cytochrome system.

*Glutathione and glyoxalase.*—The observation, recorded by the German biochemist, Lohmann, in 1932, that glutathione served as specific co-enzyme for glyoxalase greatly excited Hopkins's interest in that enzyme. He was evidently struck by its wide distribution in nature, and desired to know whether the same was true of glutathione. In his first paper on this topic, "On the Distribution of Glyoxalase and Glutathione," with E. J. Morgan,<sup>130</sup> in 1945, he writes:

The experiments to be described were directed to determine the presence or absence of glyoxalase and glutathione in such living organisms as have not been previously examined from this point of view. Our results added to those published indicate an exceedingly wide distribution of these cell constituents, especially of the enzyme. On the animal side we have dealt chiefly with invertebrates, which have been but little explored. Dakin & Dudley (1913) stated that glyoxalase is present in the oyster, and Lohmann (1936) found it in the muscle of the octopus. We have been unable to

find in the extensive literature on the subject any other similar reference to invertebrates. We have also proved the presence of glyoxalase in several plant species in which its presence has not been previously shown. . . .

The results of the experiments described add considerably to the knowledge of the distribution of glyoxalase. We have given special attention to invertebrates, which have been very little studied from this point of view. We have found that the enzyme is present in all but one of the sixteen different species from eight different orders examined. It proved to be present in a mould (*Penicillium notatum*) and among algae, in red and green seaweeds, and also in fungi. In addition, we have shown its presence in nineteen of the higher plants not previously examined. These results, added to the cases described in the literature, show how extremely widely distributed is this enzyme in living systems.

The effect must here be considered of the varying concentration of glutathione, the apparently essential concomitant for enzyme activity. . . .

It is quite certain that a deficiency in glutathione may become a limiting factor in the activity of the enzyme. . . .

With present knowledge it is, of course, generally assumed that the sole activity of this enzyme is to convert substituted glyoxals into their corresponding hydroxy acids. The absence of satisfactory evidence for the occurrence of such a substrate in the cells and tissues studied has involved disappointment to those interested in the enzyme; this will not be lessened by knowing that the enzyme may exist in certain cells and yet be unable to exert its full activity owing to a deficiency of glutathione.

This failure to gain reliable evidence for any definite function for glyoxalase has now lasted for the thirty years which have elapsed since the discovery of the enzyme by Dakin & Dudley (1913). The realisation of this may at the present time be discouraging further work on the subject. . . .

A second paper,<sup>132</sup> published in 1948, after Hopkins' death, records the separation and purification of a new factor which increases the velocity of the reaction when methylglyoxal is the substrate:

The experiments to be described took origin from a personal observation by one of us (E.J.M.). He found that the enzyme glyoxalase was well adsorbed on calcium phosphate gel at pH 5-6, the filtrate showing no activity at all in the presence of methylglyoxal and glutathione. When, however, a proportional amount of the filtrate was added to the eluate from the calcium phosphate the activity of this was largely increased. . . .

It will be seen from the results of the experiments described above that when methyl- and phenylglyoxal are added to a preparation of glyoxalase as ordinarily made from a tissue, the velocity of the reaction with the former is often more than three times that with the latter substrate. . . .

Lohmann (1932), in his experiments showing that glutathione was the

co-enzyme for glyoxalase obtained this effect with either (he did not state which) rat or rabbit liver.

There seems to be no doubt that this increased velocity, when methylglyoxal is the substrate, is due to the new factor which we have separated and purified. We have shown that when the enzyme and factor are separated, the velocity of the reaction with the enzyme alone is the same for both substrates. But when the factor is added to the enzyme it is only the methylglyoxal which shows increased velocity, the phenylglyoxal giving the same evolution of  $\text{CO}_2$  as for the enzyme alone. This is shown to be due to the fact that, although phenylglyoxal combines with the factor, such combination does not increase the velocity of reaction. . . . We have emphasised earlier that the essential effect of the presence of the factor is an accelerative one.

*Synthesis of glutathione in germinating seeds.*—Yet another study of glutathione, by Hopkins & Morgan,<sup>127</sup> refers to its appearance in pea seedlings during the early stages of germination:

Two interesting papers have been published containing studies of the phenomena with which the present research is concerned. Firket & Cornhaire state that while absent in the dry pea, glutathione, or at least  $-\text{SH}$  compounds, rapidly appear after these are placed under water. These sulphhydryl compounds are distributed throughout the cotyledons, seeming to prepare the conditions for the growth of the embryo rather than being produced under its influence. . . . Vivario & Lecloux confirm the early appearance of glutathione during hydration. . . .

The facts revealed by the above authors are of much interest, but the numerical data they present require revision. In each case it was, of course, realised that other reducing substances beside thiol groups might be present. . . . It is now known, however, that the use of nitroprusside as an indicator gives unreliable results. Moreover, these results were calculated as glutathione when this was still supposed to be a dipeptide. . . .

We have ourselves succeeded in isolating glutathione from germinating peas by methods which will be described. . . .

Kozlowski was the first to attempt the isolation of glutathione from peas. So far back as 1926, working in the Biochemical Laboratory at Cambridge, he spent much labour on the problem, employing as much as 25 kgm. of material for each extraction. He succeeded in proving the presence of non-protein cysteine but obtained no product identical with glutathione. After Hopkins had described the use of cuprous oxide as a precipitant, he returned to the problem, but again was not successful in isolating a pure substance. . . .

### *Pterins*

It was in 1942 that Hopkins' paper, "A Contribution to the Chemistry of the Pterins",<sup>124</sup> appeared in the *Proceedings of the Royal*

*Society*, to mark his brief return to a field in which he had been active some 45 to 50 years before (cf. p. 44 of this volume).

His historical introduction to the paper is of peculiar interest:

More than 50 years ago (Hopkins, 1889) I first called attention to the circumstance that a yellow pigment in the wing scale of butterflies belonging to the family Pieridae, and highly characteristic of that group of insects, is freely soluble in hot water, while preparations of the extracted pigment give a characteristic murexide reaction suggesting a relationship with uric acid. I also referred to experiments which seemed to show that it might be related to a yellow product, which as Wöhler showed in 1853, is formed when uric acid is heated with water in sealed tubes at 100–140° C. These were the first observations to be made on the pigments which have now come to be known as pterins.

Some years later (Hopkins, 1895) I was able to publish a fuller account of the yellow pigment, and I then described experiments which had convinced me that the wing scales of white pierids, e.g. of the common garden white butterfly (*Pieris brassicae*), contain uric acid itself.

These publications evoked no experimental work by others till nearly 40 years after the appearance of the first of them, and 30 years after that of the major paper. In 1925, however, Heinrich Wieland, with members of his school, began a series of researches on these substances which are remarkable for the enterprise displayed in obtaining the great numbers of insects which proved necessary for effective studies, and for the long-continued efforts made by many workers to determine the constitution of these pigments. Meanwhile, during recent years these efforts, devoted to a problem which might seem to be remote in its bearings, have apparently been justified from facts revealed in a biological field of inquiry. Thus Koschara has separated from human urine a pigment which, on what seems to be satisfactory evidence, is a pterin, closely related to and possibly identical with the xanthopterin of butterfly wings. This substance is present in mammalian tissues and may possess physiological functions, sharing for instance with the flavines an influence in tissue oxidations (Koschara, von den Siepen & Alared, 1936, 1939, 1940). Jacobsen (1939) has shown that the so-called argentaffine cells of the intestinal epithelium contain a yellow pterin, while the localisation of these cells agrees with that of the substance active against pernicious anaemia. Such coloured cells have been found absent in cases of that disease, and Koschara states that in such cases there is an increased excretion of uropterin. It is by no means sure, however, that pterin itself is active in preventing clinical anaemias, though Tschesche & Wolf (1936, 1939) make the remarkable claim that the injection of 10  $\mu$ g. xanthopterin causes a considerable new formation of red corpuscles in rats made anaemic by feeding on goat's milk, while progress of the anaemia is arrested by small daily intramuscular injections of the pterin as obtained from butterflies! If such findings are ultimately confirmed and extended,

these pigments have a wide interest, and the determination of their constitution is highly desirable. The solution of this problem proved to be highly elusive, however, and the history of progress towards it is in many ways remarkable. Recently it has become even dramatic. After 14 years of experimental labours (1925-39) provisional conclusions had been reached which, during the earlier months of 1940, were shown to be illusory. Interesting syntheses now seem conclusively to have proved, in the case at least of two of the more important pterins, that they have constitution, differing from, and much less complex than, those previously assumed for them.

In a recent return to this field, on limited lines, after 45 years, I began experiments which I hoped might throw some light on the constitutional question. This possibility has perhaps lost its interest to-day when that question seems settled. The results I have to record may, however, be found to have some interest of their own, though many of the suggestions they carry could not be followed up from inability, during recent years, to obtain the requisite material. . . .

Even the briefest reference to the earlier among the many German publications would be impossible here, but enough may be said to make the present position clear. . . .

The next section deals with "rhodopterin (formerly 'lepidoporphyrin'), a red-purple derivative from yellow pterins":

In 1895 I called attention to the fact that yellow pigments from the wings of pierids when heated on the water-bath with 20 per cent. sulphuric acid yield a purple derivative with high tinctorial power. This was true of the wing pigments from all the species then studied. The product, whatever its source, shows in acid solution two characteristic absorption bands identical in every case (*infra*). At that time I called it lepidoporphyrin, a name unsuitable to-day when the term porphyrin carries such definite and of late different implications. As present evidence suggests that as a derivative it still retains the main structure of a pterin, I propose in the present paper to refer to it as rhodopterin. The German authors paid little or no attention to this derivative; chiefly, I think, because this material did not reveal its quantitative importance. My recent experiments have shown, however, that it is obtained in relatively large yield, and is of special interest in that it arises from the pterins, or from certain among them, as the result of quite mild treatment. . . .

He proceeds next to describe some ~~general chemical properties~~ of rhodopterin, its elementary analysis, and its reduction by ~~hydrogen~~.

The discussion which follows, begins:

At the time when some preliminary observations had suggested that the purple products would prove worthy of study (1895), ~~little~~ ~~had~~ ~~been~~ ~~known~~ ~~that~~

still in touch with agents or representatives abroad, and I was assured that there might be no difficulty in obtaining coloured pierids in quite large numbers. Soon afterwards, however, contacts of the kind were completely interrupted, and I had to seek for supplies in this country. Many collectors have helped me, but it will be understood that the number of a given species which could be spared from private collections, and even from those in museums, could not fail to be relatively very small.

Ultimately it became clear that the study would have to be on more limited lines than I had hoped might be the case. . . .

Erythropterin is a pterin which probably plays as large a part in the coloration of the pieridae as does xanthopterin, and it is unfortunate that hitherto it has not been obtained pure. . . .

Later sections of the paper deal with "derivatives from uric acid showing relationships with natural pterins" and the paper concludes with an experimental section.

In his own summary of the paper, Hopkins writes:

The experiments in this paper have been chiefly concerned with a derivative from pterins, the wing pigments of pierid butterflies. These pigments have become prominent of recent years, partly on account of the prolonged efforts (1925-41) of continental workers to determine their nature. The progress of these constitutional studies has quite recently become dramatic, as is made clear in the historical introduction below. Moreover, one pterin at least has been shown to occur in mammalian tissues, with probable physiological functions there. From one pterin (erythropterin) the yield of the derivative with which the present study has dealt is large. From another (xanthopterin) it arises only in special circumstances which are of considerable interest. The substance gains in interest from its mode of origin. This involves an oxidation by molecular oxygen in solutions of low pH. On the acid side of neutrality its formation proceeds throughout a wide range of pH values, but not at all on the alkaline side. The properties and behaviour of this derivative (which in some respects are exceptional) are described, and among the experimental results obtained are some which offer guidance for future extension. The ground covered has been limited, however, by difficulties at the present time in obtaining an adequate supply of material. No evidence bearing on the actual constitution of the product has yet been obtained, and the paper represents what is essentially a preliminary study. It seems justifiable to claim, however, that until the structure of this derivative is determined and the mechanism of its formation made clear, the chemistry of the pterins will remain incomplete. Another section of this paper deals briefly with the yellow products which occur when aqueous suspensions of uric acid are heated in sealed tubes at high temperature. These show properties strikingly akin to those of the pterins, and in particular yield purple derivatives in precisely



Richardson (1936) found that under certain conditions uracil was essential for the growth of *Staphylococcus aureus*. Möller (1939) showed that adenine was required for the growth of *Streptobacterium plantarum*, while Pappenheimer & Hottle (1940) found adenine to be necessary for the growth of a strain of group A haemolytic streptococci, but also found that it could be replaced by others of the group of purine bases. Snell & Mitchell (1941) have studied the varying effect of several of these bases on strains of lactic acid bacilli. Robbins & Kavanagh (1942a) have described the effects of guanine and hypoxanthine on the growth of *Phycomyces*. Pennington (1942) has reported the effect of several purine bases on the growth of a strain of *Spirillum serpens*. This author has reported that hypoxanthine is effective alone for the growth of this organism, but can be replaced by an equimolecular mixture of adenine and guanine. But if either of these components is in excess of the other, toxic effects are observed.

It seemed desirable to us to study the effects of these bases on the growth of an animal tissue *in vitro*. . . .

Periosteal osteoblasts from chick embryo were used as the experimental material, and were grown in a medium of fowl plasma and embryo extract in the usual way. The experimental findings are set out as follows:

The experiments described in this paper have shown that the purine base hypoxanthine, added in suitable concentration to a medium commonly used for tissue culture, is capable of markedly increasing both the rate and duration of the tissue growth. On the other hand, the related base adenine acts unfavourably on the growth. . . .

The inquiry does not seem to have been pushed further, and we may observe the comment and note of inquiry:

The ability of the base to promote growth is certain. On the mode of its action it is perhaps difficult to decide. The fact is certainly not due to a strong catalytic action of an impurity. . . .

### *Vitamins and Nutrition*

Hopkins maintained his interest in nutritional topics to the end of his life.

For many years his mind had been troubled by the fact that later workers, using perhaps more highly purified basal diets, had experienced difficulty in repeating the findings described in his famous paper of 1912, namely, that a very small amount of milk (2-3 ml.) was sufficient to supply an adequacy of vitamins for the experimental rat. Workers in other laboratories, while not contesting the conclusion which Hopkins had drawn from his curves—the importance of

accessory factors in normal dietaries—found, for example, that milk was, in fact, a relatively poor source of certain vitamins, e.g. aneurin, and hence had to be given to the rat in relatively large amounts if it was to prevent deficiency.

There has been a general disposition to explain the discrepancy as being due to the occurrence of some degree of refection, a phenomena which was not recognised until long after 1912. Colour was lent to this interpretation by the fact that potato starch, known to be particularly favourable to refection, had been used in Hopkins' original experiments.

Hopkins, however, in returning to the problem with V. R. Leader in 1945,<sup>121</sup> does not accept this view. He writes:

In a paper published by one of us 30 years ago (Hopkins, 1912), experiments were described showing the marked effects of very small quantities of milk in promoting growth in rats on a diet which otherwise contained no vitamins.

At the time of these experiments such factors as refection and the effect of coprophagy had not been recognised, while the large number of nutritional factors now known to contribute to normal growth was unsuspected. It seemed worth while, therefore, when the opportunity arose to attempt to define more closely the conditions necessary for the milk effect. As a matter of fact, we have found little difficulty in reproducing the original results. Our experiments, however, have revealed some fresh facts and relations to which we feel attention should be called. . . .

A special feature of this work was the proposed use of filter paper to inhibit refection—a development due to Miss Leader:

In the course of this investigation we have dealt with many cases of refection, but during a period of several years we never obtained the condition except when raw potato starch was the carbohydrate in the synthetic diet. Recently, in the exceptional circumstances described in the text, we have had a few cases on rice starch. . . .

We have found that the administration of roughage in the manner described can, to a large extent, prevent the establishment of refection. The number of organisms in the caeca is then greatly reduced and the contents indeed may become nearly sterile.

Using potato starch as the carbohydrate of the diet, we have had no difficulty in reproducing the results of the early experiments published by one of us (Hopkins, 1912), but we found that to obtain a growth induced by administering very small quantities of milk to vitamin-free diets, the presence of this starch is necessary. Indeed, the growth so promoted calls for the same particularity in the carbohydrate supply as does the establishment of refection.

Nevertheless, we have obtained what seems to be conclusive evidence showing that the growth with milk is wholly independent of refection. . . .

Hopkins' interest in nutrition was an abiding one, and he was always prepared to exert himself to secure a better recognition of its importance. When the new journal *Ergebnisse der Vitamin- und Hormonforschung* appeared in 1938, it was he who contributed the foreword. Symbolic of his attachment to the subject was the fact that his last public appearance was at the inaugural meeting of the (British) Nutrition Society in 1941, when at the age of 80 he was asked to give the introductory address.<sup>128</sup> He said:

It is just half a century since I delivered some lectures to students at Guy's Hospital which were in part concerned with the subject of nutrition, and I am realising acutely with this large audience before me how greatly the subject has grown in importance and interest since those days. The teaching of Carl Voit was then still to the front, and we all tended to respect the figures of his dietary for a man doing an average amount of physical work, the 118 g. of protein with 500 g. of carbohydrate and 56 g. of fat. Voit believed that the adequacy of a diet was to be measured in terms of these three constituents consumed in right proportions. His view was based essentially on chemical considerations. At the time of which I am speaking, however, the star of Rubner was rising, and indeed had risen. For him, as you will know, the chemical make up of a diet was of relatively small importance. In the earlier days of his teaching he almost ignored it. The true criterion of adequacy was for Rubner the energy content of a diet. He believed that this standpoint was the more philosophical, involving thought on a higher plane than that of the chemist. It is, by the way, noteworthy that energy considerations had entered but little into biological thought up to that time.

The only other writer to whom we had to give attention in our teaching at the time was Pflüger. He was chiefly concerned to emphasise the outstanding importance of protein as a foodstuff which alone could serve *all* nutritional purposes. His attitude was unreal, and his views did not help progress. Such were the materials on which we had to base our teaching half a century ago. We strove to combine and balance them so as to present some consistent doctrine concerning the needs of nutrition, not knowing how ignorant we were of essential details. How much more we have to teach our students to-day!

Studies of nutrition were, of course, continuing at that time. They were largely concerned with the quantitative needs for protein and evoked controversies which to-day have not wholly ceased.

I have ventured to remind you of these old days and old views because remembering them gives emphasis to the great progress involved in the attainment of our present knowledge. A good many years had to elapse

before the revolution came which involved the realisation that the factors necessary for ideal nutrition are numerous and so highly specific. Like most revolutions it was resisted by some. Thinking of the conception of vitamins in particular, I recall how at a meeting of the British Association three stalwart Scotsmen united in pouring scorn on such fanciful ideas, though one of the three is now among the most influential of diet reformers on the new lines.

Sure it is that the study of nutrition has now reached a stage which abundantly justifies the founding of this Society.

Current studies and their practical application call for mutual aid among individuals with diverse qualifications, and it is a high merit of the Society as constituted that it will bring such together. I am thinking in particular of laboratory workers and clinicians, each of whom will learn much from such contacts, often, to say the truth, from mutual criticism. Practical dieticians, who have seldom themselves been investigators, will profit from hearing new additions to knowledge described at first hand, and sometimes perhaps by hearing enlightened criticism of accepted views. The existence of a Society such as this, promoting discussions to which all these and others can contribute, cannot fail to hasten progress, and our gratitude is due to those whose enterprise has led to its foundation. This large attendance at its first meeting seems to assure its future success. In the circumstances I shall not, I think, be thought impertinent if I offer it my blessing!

Hopkins, moreover, did not shirk the social implications which had been raised by the newer knowledge of nutritional requirements. He recognised that malnutrition was more prevalent than was commonly accepted, and that it was an avoidable social ill; and he lent his support to such organisations as the Children's Nutrition Council, of which he was President. On occasions he was even ready to write letters to *The Times* in support of measures of reform and alleviation, and they were not always accepted.

### *Social and Political Themes*

As Sir Charles Sherrington observed in an obituary tribute\*:

On Hopkins' 80th birthday he gave a talk in a small room—he was at his best in a small room—in his laboratory to pupils working there with him. The subject was his own career and what he inferred from it. I fancy that after biochemistry his greatest interest lay in socialism; his views were quite far to the left. He had associated with Ramsay MacDonald in the early days of both of them.

In truth, Hopkins had a deeply progressive social conscience though

\* *Lancet* 1947, (1), 728.

he played little part in active political life. His Foundation Oration\* at Birkbeck College in 1936<sup>114</sup> expresses well his social outlook.

Reference has been made above to the Children's Nutrition Council. In his presidential address Hopkins said:

I am myself old enough to remember days when respect for the doctrine of *laissez faire* was still dominant in the minds of the majority, at any rate in industrial and commercial circles 'with which I had most contact in my youth. Individualism was not perhaps at its worst in the days I remember, for philanthropic movements making for the benefit of the submerged classes were beginning, but they were sporadic, wholly inadequate, and motivated by charitable impulses rather than by any recognition of human rights. But only a decade or so previously the belief in *laissez faire* had been complete, and even charitable enterprises of any kind were condemned by many.

The industrial revolution which brought wealth and power to the middle classes was associated with a code of morality among those who were its leaders which appears the more remarkable the more one thinks about it. In mid-Victorian times the rectitude of this particular code was unquestioned by the majority. That able French observer, writer, and student of the English, André Maurois, has said of these Victorians: "The invention of the steam engine and industrial machinery, and the astounding development of English railways and mines had inspired in them a passionate belief in material progress. The new science of political economy had taught them that the relations between men are not moral relations or duties but are decreed by laws no less real and inevitable than the law of gravity or the movement of the stars. The law of supply and demand was the gospel of these men, and Manchester their Holy City." Yet the majority of these consisted of highly respected and self-respecting men, devoting one day in seven to a religion the tenets of which directly contradicted the teachings of the code which guided them in practical affairs. It was, above all, this contradiction which made their outlook so remarkable, so worthy of being recalled and studied. Their attitude towards those who worked for them was painfully illustrated by their opposition to the Factory Acts, and by their conviction that education of the wage-earners was something dangerous and even evil.

It was but natural that these men should have been actuated by the profit-motive, but as professors of a merciful religion they might well have believed that an enterprise should not begin to count profits before the demands of each social duty have been paid for as well as its material costs. Had they visualised, for instance, the machine as an agent which, while still accelerating profits, might yet soften the lot of labour, and had they from the first realised that if insecurity for the worker is inherent in the industrial system, then unemployment insurance of some kind should be a logical

\* Reproduced in full below, p. 290.

necessity and a natural part of the system, then, though wealth might have accumulated somewhat more slowly, there would have been less suffering in the past and a better balanced society to-day.

It is well I think sometimes to remember that the days of such views are after all not so far back in social history, and we have to recognise that they are yet held almost in their integrity by men of our race.

Yet years ago we led the world in sanitary reform, our pre-war social reforms astonished other nations at the time, and it must be admitted that though so inadequate they have done much towards social betterment. That improved nutrition for the people is as urgent now as was sanitary reform a hundred years ago, is a conviction slowly growing, but not yet as complete as it should be. It is easier for the comfortable classes to realise the necessity for, say, unemployed insurance in a general sense than to understand fully what an indispensable part adequate and right nutrition plays in the maintenance of health and happiness. Unfortunately, the type of education which the upper and governing classes have usually received (at least until quite lately) leaves them ignorant of the most elementary facts of physiology.

I would now like to deal briefly and quite at random with one or two problems of the moment. First with that of school feeding, with which the Council has been much concerned, and on which Miss Green has written so ably and so convincingly. A point that I am tempted to emphasise in this connexion is the extremely unsatisfactory state of affairs in many country districts. I know personally, for instance, of one village with thoroughly insanitary school buildings, where there is no accommodation or equipment for the serving of meals, and where the local magnates, whose influence is paramount, are unsympathetic and hold retrogressive views. Here there is no pretence of real concern with the nutrition of the children in the district, and I am assured that such cases are not uncommon. As bearing on this matter as a whole one is struck with the undesirable consequences which follow from the great disparity in the activities of local authorities, borough and county councils and other responsible bodies. Some few are keen, many are apathetic, and some are antagonistic. The spending power of some is adequate, others are inhibited by lack of funds. While this country is entitled to be proud of its local government organisation as a whole, it seems to me sure that the proper nutrition of its future citizens is a matter calling for a policy which is uniform, administered centrally, and supported by the national exchequer. The special question of the milk supply I dare not deal with here. The present position as regards control of production, the fixation of prices and the methods of distribution has become so complex that it calls for prolonged discussion or none at all. I will only remark that existing policy is grudging and illogical.

An inhibitory factor, which has to be faced by all who wish to see rapid progress in these matters, is the frequent demand for a proof of the existence

of malnutrition in the form of a test or tests which will confirm its existence in individuals. The desire for such exact information is natural enough, yet to those who have given adequate thought to the matter a demand for it seems illogical. We all know that a dietetic deficiency when sufficiently complete may produce unmistakable symptoms, but even a number of deficiencies, each of which amounts only to inadequacy, may exert a more subtle influence which slowly but surely undermines health. This is true in infancy, and it is roughly true that the sooner the deficiencies are suffered the more permanent their effects. An individual at a given moment may not show grave signs of malnutrition, but what the examiner cannot tell is what the same individual would have been like had he or she been certainly well fed. At any rate many inquirers are just now endeavouring to establish some definite stigmata of malnutrition, and not without some success. I think, too, the last few years have seen a change which will ultimately lead to the disappearance of this difficulty. For a long time most clinicians had small faith in the importance of qualitative faults in food—in the importance of individual vitamins for instance. That period is past and the medical literature of the world is full of observations taking account of such factors. Gradually we shall learn much more about the less obvious signs of bad feeding.

With regard to information of the kind which can be gained from a study of family budgets, perhaps not all here are aware of the ambitious investigation which, with endowment from the Carnegie Trust, is proceeding under the auspices of Sir John Orr and Dr. Magee, of the Ministry of Health, with the co-operation of the Minister. It is likely to be more profitable than some of the many investigations of the kind already made. It is to include 2,000 families, but what is more important, a considerable proportion of these is being arranged in groups, one set being adequately fed, while for a time at least the other set will not be so fed. A comparison will then be made. As trained observers are assisting, the information ultimately gained should be very valuable.

I will touch finally on great but difficult questions. I mean the possibility of establishing an all-embracing national food policy. Are we justified in thinking that this is possible? Is it possible to-day? Granting its ultimate possibility, on which lines should it be developed?

As you will know, our sister organisation, the Committee on Malnutrition—the work of which we all welcome and admire—has boldly faced this problem, and has discussed it from many angles in an able Memorandum. The Committee has not underestimated the many great and diverse difficulties involved in it, and does not, I think, intend to recommend any immediate action. Its intention is rather to stimulate public thought about the matter, and by marshalling many relevant facts to help the country to make up its mind on a subject so important for its ultimate welfare.

Few I think will doubt that it would be logical enough to remove essential foodstuffs from the crude operation of the law of supply and demand, but

we have to realise that to do this effectively would call for considerable readjustments in our social structure. It is conceivable that in the course of a few years administrative action might bring about an equilibrium between the production and importation of the right foods and a consumption right in kind and quantity, without serious dislocation of existing customs. It is different, however, in the case of the elaborate machinery involved in distribution; yet success in a national effort would certainly call for much simplification in this. Innumerable surplus middlemen and retailers, with their heavy costs, would have to disappear, and as the Committee remarks, all would hesitate to support a policy of sweeping changes that might produce widespread suffering, even if the retailers could be to some extent absorbed elsewhere.

I have always felt myself that any fundamental change in the nutrition of the peoples must involve international readjustments, and for these, alas, the times are far from favourable, even as they are unfavourable for a large increase in public expenditure. Yet we must never forget how infinitely desirable is a wise and carefully thought out national policy; one which would make malnutrition a thing wholly of the past.

Meanwhile, while waiting for some great opportunity to arise, we may be proud of what has been done by this Council towards the mitigation of social wrongs. I hope that work will continue with equal or increasing success.

The report of an address to a conference ("Alcohol, Nutrition and Fitness: Medical Aspects of a Social Problem"), called by a temperance organisation,<sup>116</sup> runs:

Sir Frederick Gowland Hopkins asked first, and in particular, did alcohol itself contribute to normal nutrition? Alcohol was relatively so expensive that to put it in the same category as butter from the point of view of practical dietetics would be the height of absurdity. . . . It would, therefore, be, economically, ridiculous to suggest that fat could, in the food budget of a family, be replaced by a quantity of alcohol equivalent in fuel value. . . .

Discussing the problem in its more practical aspects Sir Gowland said that the conference met at a time when the nation, and now its Government—largely due to the efforts of an enthusiastic minority—had been made aware that large sections of the population were ill-nourished and less physically fit than they should be. There was a widespread desire to remedy that state of affairs. All knew that, whatever aid to physical fitness other measures might provide for the poor, and indeed for a large section of the wage-earning class, better nutrition was the most to be desired. . . . Naturally, among the working classes as among others, the circumstances of life called for some form of self-chosen luxury. It was nevertheless unfair, and even cruel, that propaganda, subtle, suggestive, and intensive, should endeavour to persuade the worker that his beer made him more robust and increased

the power of his muscles, thus tempting him to increased consumption and helping to salve his conscience when he knew that his expenditure on it was beyond his means.

In 1938 Hopkins became President of the Association of Scientific Workers. In his presidential address he said:

In spite of my years I remain an optimist, particularly so when reviewing the present position of the A.Sc.W. On coming back into contact with the Association after several years I am refreshed and astonished at the vitality it has gained, and at the enthusiasm and unselfish services shown by its members. The A.Sc.W. can be of real service to scientists and to the nation if this enthusiasm is maintained.

The Executive Committee justifiably claim that a great deal has been done to extend the activities of the Association and to awaken interest in its work. I would like to say that a fundamental function of the Association, but not one that requires the most publicity, is still to work for the economic status and the security of scientific workers. In the early days the Association received criticism because it was a trade union, a title of which, after all, it had no reason to be ashamed. Scientific workers have not got the advantages of the organised professions, and they must perhaps always be on a different plane. The British Medical Association has developed to a point where it can compel every employer of a qualified medical man to pay him at least £500 per annum. The public understands the worth of medical men, but even to-day the importance of scientific research is not understood by the general public. Not so long ago the attitude towards research was that it was an activity to be carried out by a teacher during his leisure, but the position is very different now. In the growth of research and in the great increase in number of research workers, society has a new problem to face, and our difficulty lies in the fact that even now many people do not realise the great importance of the calling which should call for the highest respect and the necessity for its proper organisation.

The scientific worker finds difficulty in securing a permanent career, and perhaps this is felt most in academic circles. Somewhat special is the case of those who work in fields related to medicine. In their case the sources of research grants are more numerous than in other subjects, and often a man finds that he can continue with research endowments for some years. Then, however, unless he is himself qualified medically he is left without teaching experience, and therefore not well qualified for academic posts, and no prospect of a permanent post in research. The Royal Society has long been alive to this problem and provides permanent posts for research workers of distinction. I hope and believe that it may extend this policy, but of course it can only provide for a few. A long-range policy for research in this country is urgently needed.

The Association has also its more immediate aims, which are urgent

and practical. For success we need public sympathy and public understanding, and we try to attain this by means of lectures and film shows, etc., which put forward the needs of scientists and the claims of science. It has often been said that the English as a race have few intellectual interests, and science is not one of them. As a result of the wide educational movement there has been considerable development of awakening to its importance in the last fifty years, but we are planning to make this really effective.

I think that the three National Research Councils are functioning well, but I sympathise with the desire of the A.Sc.W. to see a still wider policy for national research, and one more planned. The success that the various Research Councils and Associations have had does not make it the less true that the funds available for research at present represent a very small proportion of the national spending power, and a smaller proportion than that spent in some other countries. I agree therefore with the intention of the A.Sc.W., which is to strive for the establishment of a really generous national attitude towards the needs of research.

I consider that even the creation of a Ministry of Science is to be seriously contemplated, though the great majority of the British public, as well as members of Parliament, would be astonished at such a demand.

I would like to speak of the Press. We do want the progress of science and the claims of scientific men made better known to the lay public. It is only the sensational, usually wrong and often contemptible items that get into the press. Those of you who have heard of the American "Science Service," and read its publications, will realise that they have got the right idea, even if the way they put it into practice leaves something to be desired. The Association should work for the improvement in the standard of science news in the daily press.

### *Chemical and Biological Thought*

Hopkins' most permanent intellectual interest was undoubtedly to ponder on the chemical explanations of living processes—not only in terms of their descriptive details, but also on the broadest philosophical plane. A number of addresses, late in his life, contain his reflections on the philosophical, the historical and the "co-ordinative" aspects of this theme. In his 1936 Foundation Oration,<sup>114</sup> at Birkbeck College, already mentioned,\* he says:

One would like to believe that even science students to-day can find leisure to read a little philosophy, but I fear that very few will get down to the reading of Epictetus. On the other hand, students of this College need not go far afield to learn just how much philosophy matters to science or science to philosophy.

\* Reproduced in full below, p. 290.

The technique of scientific discovery is discussed in the address<sup>122</sup> at the opening of the Imperial Cancer Research Fund's Laboratories in 1939, which contains a polemic for the provision of better facilities for laboratory research ("The Importance of Laboratory Effort in Cancer Research"):

I have spent much of my life in laboratories, and it has been one of my main interests to try and follow the effect of that great increase in the number of laboratory workers which has occurred in my time, not only in this country's contribution to scientific knowledge as a whole but to medical progress in particular. My memory goes back, doubtless like those of some others in my audience, to years when accommodation and opportunities for experimental research were sadly to seek in this country. It was then the almost universal custom among young men who hoped to devote some at least of their energy to scientific research, to spend an apprentice year or two abroad; usually in a German laboratory, where inspiration and adequate equipment might in those days be most easily found. For this expatriation there is happily no need to-day, but it must not be supposed that the provision of much more laboratory accommodation would fail to benefit both science and the country. . . .

This address proceeds to deal in turn with "the method of observation; the method of experiment; cancer; dogmas and facts; transmissibility of tumours; properties of malignant cells; the pathogenic agent in filtrates; some provocative factors; recent developments in cancer research; freedom, leisure, and the side-paths; grounds for a hopeful view":

. . . New constellations of illuminating facts continue to appear above the horizon of knowledge, and it is not too much to hope that at any moment some pregnant item of new knowledge may appear among them which, like a pole star, will point directly to the right path for effective action. Such a consummation may come soon or in a more distant future. Meantime, the patient efforts of investigators must continue.

A contemporary editorial comment in the *British Medical Journal* (1939, (2), 21) reveals the existence of a rival point of view:

Hopkins touches on the important point of freedom for the research worker, and shows that it is impossible to predict the direction from which great advances may come. Many people's minds are exercised by the much greater danger that research may become a closed concern and that a hard-and-fast line may be drawn between the research worker and the teacher. It would be idle to pretend that this danger does not already exist in pathology, with the growth of large whole-time research institutes such as the Lister Institute and the Medical Research Council's Institute

at Hampstead. Moreover, it seems generally agreed that not only men engaged in clinical practice but also those with no other duty than clinical teaching have insufficient time for advancing medicine by experiment, and clinical schools are growing up in Cambridge and in Oxford which have no contact with the undergraduate student of medicine. The gulf between the research laboratory and the lecture theatre or ward becomes continually more difficult to cross. . . . And it must surely be wrong for students in this country to have no living contact with the men who are making the most important advances in their subjects.

Once again in retrospective mood, in the Linacre Lecture for 1938\* ("Biological Thought and Chemical Thought: a Plea for Unification"), Hopkins dwells on the use of biochemistry during the previous half century:

In an endeavour to illustrate the kind of progress biochemistry has made in my time and, if I can, its significance, I might have chosen almost any one of very many aspects. I have decided to make reference to some which to me, at least, seem characteristic. They are aspects which, in general outline at least, are among the most familiar, yet I feel that they, perhaps more than any other, have altered my own outlook. I will refer to our realization of the fact that the phenomenon of *catalysis* is universal and fundamental in the scheme of living Nature, and further will remind you that the frank influence of molecular structure is exerted to the full in every living system. I will then ask you to remember the circumstance, now in a general sense so familiar, that the normal progress of events in every living system calls for the presence of a number of highly specific substances, each exerting its own peculiar influence in a quantity which is almost infinitesimal; implying that the chemical equilibria on which life depends may be balanced, as it were, on pin points.

Hopkins concludes with a defence of the use of "biochemical thought," as he terms it, in the description of biological events:

. . . The use of the term protoplasm may be morphologically justified, but chemically it denotes an abstraction. It is sure that it is made up of parts in which the influence of molecular structure as understood by the chemist is all potent. . . .

Let it be frankly admitted that biochemical studies have still before them, save in small, though not negligible, part, what I will again claim as their legitimate aim and major task—the endeavour namely to describe biochemical events from the standpoint of their organisation during life. If and when such effort succeeds, and only then, we shall know just how far chemical thought can reach towards a full description of any living unit. Many, and, I think, most to-day, believe that the full description will call

\* Reproduced in full below, p. 302.

in addition for thought of another kind. Nevertheless, at any one level of organisation description may have its own measure of completeness. . . .

I was a little disconcerted when asked, after the title of this lecture was announced, how I would define biological thought for my purpose, for it has many varieties. What I meant by it as applied in the particular field which I have made the special concern of this lecture, is such thought about a living unit which ignores, or minimises the importance of, those molecular events which must underlie the visible manifestations of its life; an attitude of mind often based perhaps on a doubt whether any attempt to correlate the molecular with the visible is within the competence of experimental science. Let me add that I have met the same doubt in the minds of some few highly competent chemists. Their view is that biochemistry should endeavour to follow every path that will lead to utility. Such paths are many and open; let us, they say, do what we know we can do and not follow a will-o'-the-wisp. I cannot agree with this view.

Some fitting passages to close these extracts, summarising as they do, Hopkins's point of view—his survey of the past, together with his predictions of the future—may be found in the address,<sup>113</sup> "The Influence of Chemical Thought on Biology,"\* given at the Harvard Tercentenary Conference of Arts and Sciences at Harvard in 1936:

The latter half of the last century, though a period of such rapid progress alike in physical and biological science, saw inadequate contact between the thought of the chemist and that of the biologist.

It is true, and a familiar circumstance to those with an interest in the history of science, that, when that half century began, organic chemistry and what we now term biochemistry were both yet in embryo and were hardly to be distinguished. Justus von Liebig fathered them both.

It was the genius of Liebig that started modern organic chemistry on a triumphant career, and Liebig's great desire and one which directed his own efforts was to see chemistry render full service to animal physiology and to agriculture. This desire, in satisfactory measure, was not fulfilled during Liebig's own lifetime, and it is, I think, of some historical interest to decide why, during years when scientific minds were so alert, so promising a field was cultivated by so few. At first I think certain personal attributes in leaders of thought contributed to the separation of chemistry from biology. Liebig himself, for instance, though so brilliant a chemist, lacked biological training and, as I have always felt, a biologist's instincts. When with great enthusiasm he came to apply his chemical knowledge to the living plant and animal his thought often went obviously astray, and much of his theoretical teaching was instinctively and rightly rejected in biological thought. What was really so valuable in that teaching lost therefore some

\* Reproduced in full below, p. 281.

of its influence. Strange as it may seem, the influence of that other dominant mind of the time, that of Pasteur, did not altogether favour an approach between chemist and biologist. If Liebig remained too much the chemist, Pasteur, once he entered, with such immense profit to science, the biological field, became almost too much a biologist, at least in so far as he favoured the current belief that the activities of a living organism could be understood only by thinking in terms of that organism as a whole. Any analysis of its totality he held to be of little avail. . . .

Apart from the divorce between chemical and biological thought, there was a tendency in the latter which in itself discouraged attempts to probe the secrets of living cells by chemical methods. Most biologists were content to ascribe the internal events of metabolism to the elusive properties of an entity insusceptible of profitable analysis; to the influence of protoplasm as a whole. There was, as I well remember, a widespread feeling that chemical studies which interfere with the full integrity of protoplasm could at most have chemical interest and must remain without bearing on the realities of biology. Looking back I find it interesting to recall that it was just when the last century was giving way to this that certain advances occurring together within the space of a year or two greatly helped to change a point of view which for the chemist had been wholly inhibitory. I would instance the publication of Emil Fischer's brilliant work on the chemistry of proteins, the discovery of hormones, and in particular the recognition, too long delayed, of the fact that the progress of chemical events in the living cell is controlled by definite objective agencies, the enzymic catalysts. These and other aspects of new knowledge, revealed together at a critical moment, started biochemistry in its more modern guise on a period of rapid progress which to-day is even accelerating.

Lest they should be unfamiliar to some, I will venture to put before you in the fewest possible words an appraisal of the present position and outlook of this branch of science and will endeavour to convince you that its facts are significant. . . .

What, you may ask, from the standpoint of pure knowledge is the goal of these intellectual activities and what will be their ultimate accomplishment? I have faith that in the end they will reach to a description of living systems which, in so far as they are chemical systems, may be complete. From a knowledge of individual events they will proceed to an understanding of the organisation of these events; that organisation which makes the organism. I can see no obstacle to the attainment of such an intellectual synthesis of knowledge. When that synthesis comes it will involve a full understanding of many of life's visible manifestations, which is, of course, not to say that it will define life itself.

If, however, the claim of biochemistry is to describe life, at any level, in chemical terms, it may come more under the eye of philosophy than perhaps any other branch of biology. There are schools of philosophy which will continue to ignore facts of a kind accessible to the chemist as

being without significance in their search for reality; but there are other schools which will at least take note of them.

It is sure, I think, that biochemical facts and biochemical thought will provide fresh aspects for biological thought. They will no less strengthen the ability of biological science to serve humanity.

It is sure that *if he can add to what the eye itself reveals an adequate mental picture of the invisible molecular events which underlie the visible*, the biologist will gain increased understanding of the behaviour of every living thing. The physiologist too will add to his understanding of every organic function; and the clinician, no less than the pathologist, will acquire a deeper insight into the significance of every departure from the normal. This is my faith, and I hope it may be yours.

# Sir F. G. Hopkins' Personal Influence and Characteristics

by

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## SIR F. G. HOPKINS' PERSONAL INFLUENCE AND CHARACTERISTICS

IN writing something on "Hoppy" (as he was universally known in the world of biochemists) it will be our aim to try to give some impression, however faint, of his personality, supplementing the accounts of his life and work which have already appeared.\* Our excuse may be that for more than 25 years he stood *in loco parentis* to us, as to all other Cambridge biochemists of our own and neighbouring generations.

It is, therefore, not necessary to repeat in detail the story of his life and principal investigations—his early work in a toxicological laboratory, his medical training at Guy's Hospital, his invitation to Cambridge in 1898 by Sir Michael Foster, the tutorship at Emmanuel, the praefectorship at Trinity, a chair in 1914, a great new building in 1925, the O.M., the Nobel Prize, and so on. Widely known, too, is the course of his researches; first on gout and uric acid and the pigments of butterflies, then on amino-acids with the discovery of tryptophane (with Cole), then on the accessory food factors now called vitamins, next to muscle contraction and lactic acid (with Fletcher); and, finally, to oxido-reductions and the discovery of glutathione—a subject which kept his interest during most of the period between the two world wars—until at the end he returned to his earliest interest, the pterine pigments of lepidoptera, now with much wider significance and more precise analysis.

The first 25 years of Hopkins' work and teaching were carried out in an extremely old and uncomfortable building in Corn Exchange Street, many of the rooms of which were semi-cellars. We can well remember this, as it lasted in partial use until the construction of the present Institute in Tennis Court Road in 1924. Much of the equipment was antique, too, for example, a centrifuge which, mounted on boards and having no arrangements for attachment to a base, used to ride all over the floor of a cellar when in operation. It was in these rooms that the rat-colony was kept; its entire feeding and upkeep during the classical investigations on vitamins in 1910 were done by Hopkins himself. Upstairs was the shelf on which had stood in the

\* *Brit. Med. Journ.*, 1947, (1), 742 (by Sir Henry Dale); *Lancet*, 1947, (1), 728 (by L. J. Harris); *Nature*, 1947, 160, 44 (by M. Dixon); *Biochem. Journ.*, 1948, 42, 161 (by M. Stephenson); *Obit. Notices, Roy. Soc.*, 1948, 6, 115 (by H. H. Dale); *Journ. Chem. Soc.*, 1948, 713 (by E. Mellanby).

sunlight the bottle of acetic acid which had led to the discovery of the glyoxylic test for tryptophane. Below were the ancient benches on which "Charles"\* had cut up the quarters of horses towards the end of World War I for the extraction of tryptophane.

The year 1920 saw expansion into a building hardly less queer than that in Corn Exchange Street, namely, an old chapel in Downing Place, where the space level with the galleries had been specially floored. A bridge connected the main part with an annexe in which Hopkins himself worked. Here developed all the lines of research which were afterwards to characterise Hopkins' Institute—intermediary metabolism, especially that of sulphur compounds; bacterial metabolism; the chemistry of muscular contraction; the precise physico-chemical study of enzymes and pigments; embryological and zoological biochemistry.

By no means the least important of the rooms in this building was the library (Hopkins was always ready to die in the last ditch for the departmental-library principle) and tea-room. Throughout the 50 years of Hopkins' work at Cambridge there were held weekly or fortnightly tea-club meetings, at which one or other of the research workers would give an account of work in progress or nearing completion. The standard of criticism at these meetings was extremely severe, so that the speaker met with more rational and constructive comment there than he was ever likely to encounter at subsequent meetings of public societies to which he might communicate his work. It was at these meetings that Hopkins' genius particularly showed itself; he invariably made every paper seem interesting, what the author might have dully presented, he illuminated with a flow of historical allusions and valuable suggestions springing from his profound intuitive insight into the structure and function of the living cell. No one could speak or act as if blinded by passion or prejudice when in the presence of a leader so objective yet so encouraging, so affectionate yet so penetratingly quizzical, whose insight was conveyed by hints and odd remarks to which no one could object, but which one disregarded at one's scientific peril.

Perhaps these meetings were so searchingly critical precisely because of "Hoppy's" own extraordinary charm and kindness. Under his aegis almost anything could be said without fear of offence. This was, in fact, his most outstanding personal quality; he was a living embodiment of the Confucian maxim that one should behave to

\* William J. Jolley, who served the Departments of Physiology and then Biochemistry for some 40 years until his death in 1936.

everybody as one receiving a great guest. The humblest laboratory assistant or the youngest research worker was always sure of a welcome, and a hearing much longer than he was likely to get from any other scientific man of the same standing or generation. Hopkins had faith in people. Colleagues were known to remark lightly, "All Hoppy's geese are swans," but they forgot that there is an induction process by which certain geese may be turned into swans if given the hormone of encouragement.

It was undoubtedly for these reasons that when we attempted recently to recapture some characteristic sayings of Hopkins, some "famous remarks" of Johnsonian flavour, we found it quite impossible to do so. This fact is of interest in itself. He was too courteous, in a way too self-effacing, to allow himself to be the author of epigrams which might hurt anyone; he expressed himself so diffidently, and above all, he spent so much of his time listening to other people, that his essence cannot be treasured in a few *bons mots*. He was a great giver and receiver of moral support—if he gave freely of it to all his collaborators and colleagues, he also needed it, for he frequently passed through periods of depression. And this was one of his secrets, that he was open to receive it from the most junior of his research workers, so that they did not feel he was encouraging them like some *deus ex machina*, but as one of themselves; in other words, he fully understood and practised the great doctrine of leadership *from within* and not from above.

Anyone who obtained the impression from what has been said that Hopkins had a colourless personality would indeed be wide of the mark. He did not say things that make the stuff of anecdotes, he did not speak much on the radio or appear prominently in the public press, he did not engage in polemics. Moreover, he had the knack of surrounding himself by people of striking personality, such as A. v. Szent-Györgyi (the Hungarian Nobel Laureate and discoverer of the chemical constitution of vitamin C), J. B. S. Haldane (afterwards Professor of Genetics at London), Marjory Stephenson (one of the first women to become F.R.S.), T. S. Hele (later Master of Emmanuel and Vice-Chancellor of the University), etc. etc. But nothing ever put "Hoppy" in the shade. No one could fail to recognise in the little figure, rubbing its eyes in a characteristic gesture during a conference, loitering with its overcoat unbuttoned in the hall, proceeding with ruminative walk past the colleges, the authentic gold of intellectual inspiration, the *Fundator et Primus Abbas* of biochemistry in England.

The pattern completes itself when we look at the various practices which he established. Contrary to what has been known in some English and many Continental laboratories, Hopkins would never put his name to any scientific paper for which he had not himself done a considerable part of the manual and experimental work involved. Although no doubt great results are to be achieved by the direction of large teams, he was never interested in forcing the diverse talents of his younger colleagues into the funnel of one problem, as he could well have done in such matters as vitamins or oxido-reductions. On the contrary, he was always looking out for originality, and encouraged the smallest sprouts of it to develop in their own way. Sometimes a young worker would devote himself to some subject which "the Professor" had initiated and then dropped, and make a life-work of it—this happened with vitamins, with muscle biochemistry, and with oxidation-reduction enzymes. In other cases, a young worker would start something new with every possible encouragement from Hopkins—this happened with the metabolic chemistry of micro-organisms, with embryological biochemistry, and with the study of plant pigments. One of the writers certainly will never forget how quickly Hopkins appreciated the fascination of the hen's egg as a closed-box system of chemical transformations; he said that there were many lifetimes of work in it, and it put him in mind of a discussion he had had at Michael Foster's breakfast-table some 20 years earlier.

If, then, Hopkins's outstanding personal characteristic was a universal courtesy, even a capacity to suffer fools gladly (knowing that some fools may change into wise men in the process), what was his outstanding intellectual characteristic? Originally, we believe, it was a deep intuitive faith in the explicability of the biochemistry of the living cell—the same faith which once inspired Aristotle when in the face of the apparently infinite complexity of living forms, he nevertheless maintained that it was possible to classify them, and proceeded to do so in at least a preliminary way. Having in mind what we know now about the numbers of species of arthropods, and how little we yet know about the physiology of invertebrates in general, Aristotle's faith seems indeed extraordinary. Hopkins was of the same lineage, for at a time when it could be said, or had until shortly before been currently said, "*Tierchemie ist nur Schmierchemie*," he knew better. He was not only an organic chemist who could isolate substances from living tissues and study their chemical structure; he was a *bio*-chemist who could in imagination perform the synthetic process of building back the living cell and of visualising

what processes were going on in it.\* And this was all the more extraordinary in that he was himself not the direct pupil of any of the pioneers of biochemistry. Here came into play his intuition. At a relatively late period one of the more provocative of the younger research workers opined that "Hoppy" was not a great experimentalist, and on being asked why, replied that it was because he did not need to do experiments, he knew which way the cat would jump beforehand, and only needed to carry out practical work to demonstrate what his insight had already told him was true. This was not at all an unfair judgment, though paradoxical in that Hopkins was, in fact, a brilliant practical chemical manipulator and experimentalist; a most accomplished laboratory worker. By 1914 Hopkins had transmuted the old German jibe into the famous definition "Life is a dynamic equilibrium in a polyphasic system."

It is necessary to emphasise the remarkable quality of Hopkins' creative imagination. Sir Charles Sherrington brought this out when, in 1947, he wrote:

I have memory of an address given by Hopkins at a British Association meeting. In an uninviting gaunt lecture-room he descanted for nearly an hour on the cell as a theatre of chemical processes. Without any deliberate attempt at eloquence, and in a voice that as he became more interested fell to such a purely conversational level as to be a little difficult to hear, he conjured up for his audience—some 30 persons all told—a picture of the cell as a tiny sponge-work containing perhaps a thousand foci of different actions co-operatively confined within a unitary whole. An organised factory manifoldly hydrolysing, pulling to pieces, and contemporaneously constructing and reconstructing. And this unity bounded outwardly by a mosaic of countless chemical poles and leaking like a sieve. As I listened I felt I was being privileged for the time to see something of the micro-cosmic world in which my friend's scientific thoughts took shape and did his bidding. One mental factor which, it seemed to me, such thinking must demand, was a peculiar intensity of visual imagination, continuously checked in factual knowledge. The great organic chemist, conjuring with stereographic formulae, must have something of the same faculty. It is lived with. Kekulé hit on his famous key-formula when riding on a London bus.†

As to the scientific literature, Hopkins was by no means a despiser of it, as some eminent experimentalists have been, trusting in their own originality and in the mistakes of others. He read a great deal,

\* Cf. for example the revealing phrase "substances undergoing *comprehensible* reactions" in his memorable Birmingham address of 1913; see p. 137.

† *Lancet*, 1947, (1), 729.

and often inspired his listeners with enthusiasm for neglected pioneers such as Thudichum, the investigator of the brain lipids; or de Rey Pailhade, the discoverer of "philothione," the hypothetical substance which preceded the real glutathione. In later stages, the walls of the laboratory were decorated with engravings of Lavoisier, Harvey, Boyle, Hales, Scheele, etc., together with an original manuscript of Liebig. Hopkins' lectures to the advanced class were often largely historical. One characteristic which all his pupils remember is his trick of waving about in a beaker of water the feather of a tropical bird, from which the water-soluble pigment turacin comes out—anyone who was not interested in such a phenomenon would, he used to say, never make a biochemist. It was equally characteristic of him that when, on one occasion, one of the more phlegmatic young research workers was not perceptibly excited by it, he remarked that of course one must in this test make allowances for temperament. At the very close of his life, when he was perhaps remembering more of his youth, he told another pterine investigator that as a boy he had taken the wings of a butterfly and heated them in a spoon on the kitchen range of his home; when the pigments dissolved in the water he was extremely astonished and delighted.

So far as we can remember, Hopkins had no particular hobby, except reading, which he did largely in a fairly unselective way, on all kinds of subjects, such as history, archaeology, and the like. One of his daughters was destined to become a distinguished archaeologist in her own right, doubtless not without some encouragement from these broad interests of her father's. Though he never visited any of the remoter parts of the world, he always took a great interest in people who came from them, and welcomed them to his laboratory, which thus acquired and maintained throughout a thoroughly international character. Before the First World War he had a number of Japanese pupils, but later only one—an odd character who caused some confusion in the laboratory by coming in late at night to work (it was typical of the Institute that it was always available for work all night) and hanging his coat and hat on the thermostat rod of an incubator. China and India were represented and North and South America very much so, while all the countries of Europe and the Near East contributed at one time or another their quota of investigators, as did all the Dominions of the Commonwealth. Frequently a postcard would arrive filled with signatures from bodies such as the "Hoppy Club of San Francisco." Correspondingly, the presentation volume, *Perspectives in Biochemistry*, which he received upon the occasion of

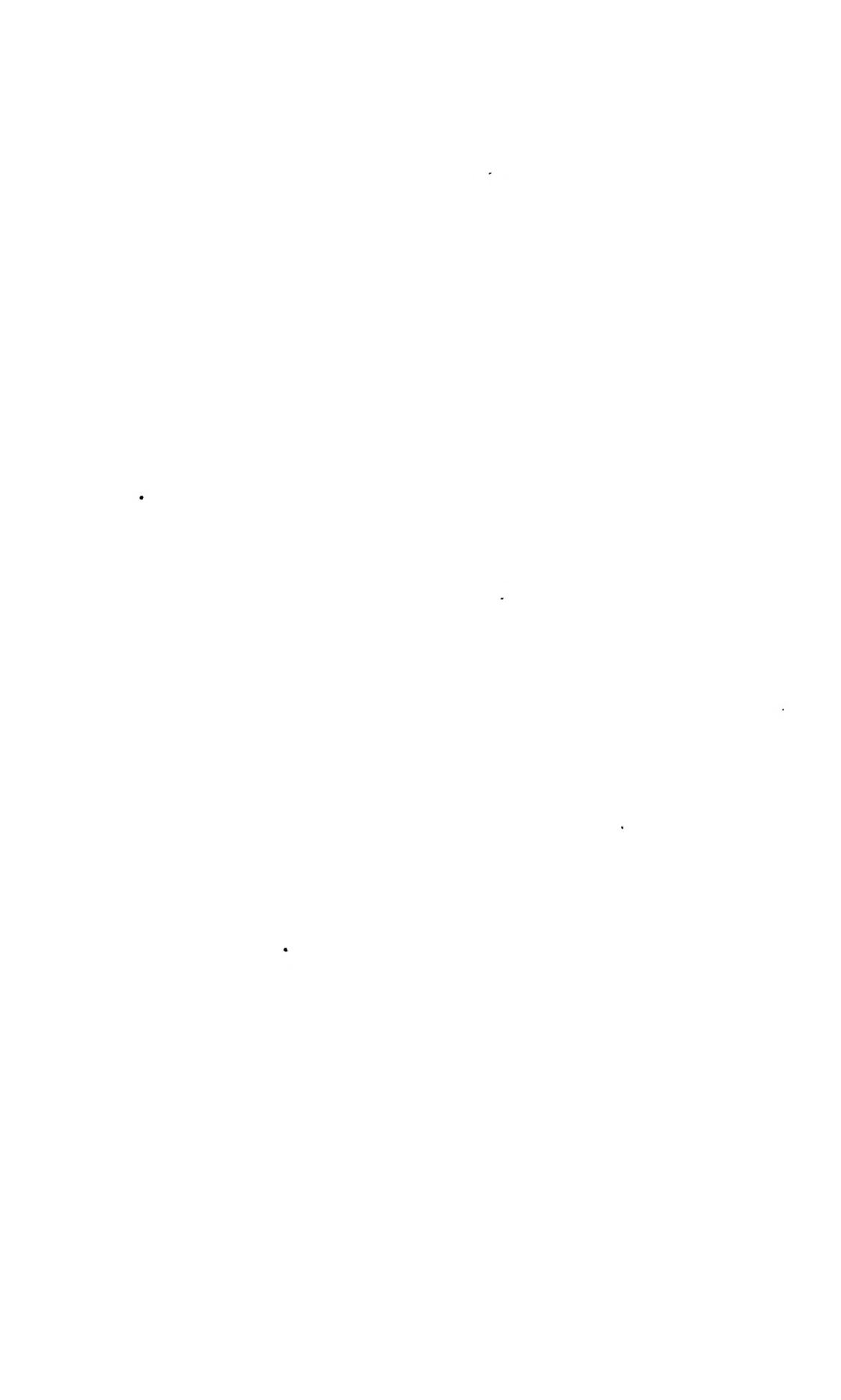
his 75th birthday, was a success altogether unexpected by the Cambridge University Press, and went into three editions.

From what has already been said, it will be obvious that Hopkins was naturally a democrat. We shall always remember a meeting, held in 1942, which was designed to inform all those working in the Institute, including especially the laboratory attendants and cleaners, in semi-popular language, of what exactly were the various pieces of war research being carried out in the Institute. Each speaker was given only three minutes. It was expected and understood that the Professor, in view of his age and eminence, would take longer; but no, the Nobel Laureate and ex-President of the Royal Society said his say in exactly the same time as all the others. The meeting achieved its purpose of intensifying the war effort of the Institute.

A perfectly sympathetic personality is not usually achieved without cost. There is no doubt that Hopkins had a very severe struggle in his early years, and at the end of his Emmanuel tutorship he was so overworked that he suffered a kind of nervous breakdown for a short time. Daily, hourly, as everyone must with something new to give to the world, he battled with the philistines. Unfortunately, in his very last years he also had much to suffer, when increasing physical disabilities co-existed with undiminished retention of intellectual power and curiosity. But it all resulted in a personality which drew from his colleagues not only intellectual admiration but deep affection and even veneration. So many of the Taoist paradoxes were found to be applicable to Hopkins—"The Sage follows after things, in order that he may control them"—"The Sage has no personal wishes, therefore all his desires are fulfilled." Inspired by his intuitive understanding of the living cell, Hopkins stood self-forgetful in front of the facts. One can see him now, at the top of the stairs, coat open and hands in pockets, backed by his technical assistant with cigarette drooping over the centrifuge tubes; head quizzically slightly at an angle, benignly smiling, inquiring about the latest results. One never came across anybody at all like him, and now one is sure one never will.



# Selected Addresses of Sir F. G. Hopkins





my case is doubtless not unique, but if anything worth saying can be said about the relations of these two professions, the circumstances, at any rate, give me a claim to speak without prejudice. With no other endowment than what I have indicated, I venture, for lack of other text, to say a few words on the relations, present and future, between the analyst and the medical man.

There is no need, I think, to repeat the indisputable statement that the doctor and the analyst share between them—not forgetting the work of the sanitary engineer—almost the entire burden of the maintenance of public health. It would be but repeating the obvious, moreover, to say that these two professions, having such common aims, should possess full sympathy and mutual understanding. But although we may believe that the sympathy is always present, yet the understanding is not always quite complete, and the lack of it has, now and again—we rejoice to know, not often—led to some friction at points of contact. When such small differences arise it must be admitted that the medical profession has this great advantage over yours—that its claims and its merits are so much more easily understood by the general public. This, should a need for judgment arise, somewhat unfairly strengthens the hands of the one disputant. It must, unfortunately, be recognised that, even now, after all the years during which the Public Analyst has served the community, the layman has a most imperfect knowledge of what his work entails. The services of the engineer are understood, those of the doctor are appreciated, but the skill expended in the analytical laboratory is understood and appreciated but little. This, to you, is again a commonplace; but I believe that I am justified in adding to it the really regrettable statement that even the average medical man is not greatly different from the man in the street in this respect. He has at one time known a little chemistry, but he possesses no standard to measure what is required of the skilled analyst. It should be understood that I speak of average cases only.

I am going to take the risk of making a bold generalisation here which I must justify afterwards. I believe that, of those professional men who in any sense apply science to practical affairs, the average doctor is among the least scientific, while the professional chemist is among the most *scientific*. I hope I am not making this statement gratuitously, and I am certainly not urging the contrast to ingratiate myself here, or to cast a slur upon the medical profession. There are many in the latter who are using high scientific knowledge, and applying it in the most difficult of all regions, the treatment of disease; but nowadays the medical man, with a real scientific bent, tends to cleave off from the profession, as a pathologist or physiologist. It is even the fashion, once more, for distinguished medical teachers to urge upon their students that the art rather than the science of their calling is the important thing; and we must all, indeed, recognise that in practical medicine, skilled and wise empiricism, based upon experience, is a much better endowment than ill-digested science. The progress of medicine



waters, I must certainly answer, No! The real value of such practical work as he does in the class is in enabling him the better to appreciate the significance of analytical data when they come before him. If he subsequently argues to himself, "I have been to the trouble and expense of learning water analysis, and I am stamped by the examiner as efficient: why should my knowledge not add to my guineas?" then, so arguing, he shows himself a man without sense of proportion.

But, remember, there are individual Medical Officers of Health who, owing to special tastes and opportunities, do try subsequently to make themselves efficient in this connexion, and it is going much beyond the facts to assert that an intelligent man pre-eminently in touch with practical problems, having carried out routine analyses of a score or so of water samples, is not in a position to claim accuracy for his work so far as it is of this routine kind. You would, if I mistake not, accept the data obtained by your own junior assistants after similar experience.

But the existence of these exceptional cases is beside the mark. To the normal medical officer, even if he be possessed of some skill as a chemist, the chemical laboratory and its pursuits are but an accident. He can enter it only seldom, and if he come into it sample in hand it is usually to find standard solutions which time and accident have made unreliable, and apparatus untested for weeks or months. Nine times out of ten, therefore, the analytical effort is a trying one, and if the medical officer spend the time necessary for accuracy in a laboratory which is not strictly a going concern, the enterprise is to him unprofitable. He should realise how much simpler it all is to the professional analyst in constant practice. No one, I venture to assert, has seen more of these things than I have, and I certainly believe that the medical officer who does not refer all the water analyses which might come his way to his chemical colleague is a person neglecting other, and to him more profitable, opportunities.

But into the controversy about water analyses there came some years ago a certain *tu quoque* argument, and to the disinterested outsider the position was even a little amusing. The examination of water is only partially complete without a bacteriological study; and though it must be admitted that our power to dogmatise about the details of contamination has not yet been aided by bacteriology to the extent that was hoped for, yet it is certain that the water expert must be, on special and limited lines at any rate, a bacteriological expert. But in bacteriological study the medical man for the most part preceded the analyst, and "Hands off!" at first seemed to him as legitimate an expression when uttered on his side of the boundary as when used on the other.

The technique of bacteriology on its cultural and merely diagnostic side is much simpler, more limited, and more empirical than that of analytical chemistry; but the former subject, like the latter, requires something more than a formal knowledge of technique. It is the development of certain special instincts, only given by long contact with the problems, which

converts the amateur into the expert. The ordinary training of the medical officer or of the analyst gives in neither case this endowment as regards bacteriology. There is, however, I believe, this fundamental difference between the intrusion of the Public Analyst into bacteriology and that of the medical officer into chemistry: the analyst is first, last, and always a laboratory man, while the medical man is not. The six years of laboratory life which must elapse before the professional chemist is looked upon as fully qualified make the attempt upon new laboratory ventures an easier task for him. This confinement to the laboratory is, on the other hand, the cause of some distinct disadvantages to the analyst. The four walls of his workshop hide him from the gaze of the public; he triumphs over difficulties in secret, and without appreciation. But he should at least reap the advantage of a recognition, from those who know, of the fact that his constant practical experience at the laboratory bench endows him with fundamental instincts for laboratory work in general, which the occasional visitor to the laboratory can only very exceptionally possess.

Some of you have doubtless been practical bacteriologists for as long a period as any pathologist can claim to have been, but the question is now rather as to how far the student who intends to practise chemistry in the future shall spend time and energy on the study of bacteriological technique. It seems to me highly desirable that a certain proportion among chemical students should be encouraged to take an interest in the subject, because of the enormous amount of purely chemical work to be done in connection with it, and because of its growing importance in subjects quite other than medical ones. These are the students for whom Branch E of the Institute's final examination, with its admirable syllabus, is intended.

But as regards the future activity of those who become officers of public sanitary authorities, it is likely that specialisation will increase and the bacteriological laboratory will become a unit. I think specialisation will here become accentuated, because of the familiar difficulty due to the intrusion of the pathogenic organism. It is, of course, not possible to draw the line sharply between the study of these and the non-pathogenic groups. The *B. coli communis* is an organism which must always concern the water specialist, and yet its case is one which bridges any gulf between the pathogenic and the non-pathogenic. But to follow up the study of the pathogenic organisms fully requires the use of animal experiments, with all their attendant difficulties. The rigour of the licence system is likely to increase rather than diminish, and only specialists, working in a comparatively few licensed laboratories, will be able to do useful work. This will lead to the emergence of the bacteriologist as a specialised officer of all sanitary authorities, and since laboratory work is best grouped in accordance with the technique employed in it, rather than in relation to its immediate aims, the specialised laboratory is likely to attract all public bacteriological examinations to its domain. This is my view of the future, and though, for reasons already mentioned, I utterly disagree with Professor

Hewlett, for example, when in a presidential address last year he declared that "the present tendency of Public Analysts"—assuming this exists—"to undertake any and every form of bacteriological work is fraught with the greatest danger . . .", yet I believe him to have been right in urging that the bacteriological specialist must emerge in the future. It is highly desirable that the knowledge possessed by any expert should extend beyond the limits of his daily task, and, in fact, that it should be as wide as the shortness of life permits; but it is equally desirable that in the application of expert knowledge to the public service, specialisation should go so far as Society and the State can afford.

If in the future the Public Analyst is to be reminded by other specialists that his business is chemistry, the medical officer will be treated on similar terms, and I have firm faith myself that an increase in the analyst's activities, on purely chemical lines, will leave him well content, as I hope in some sort to show later.

There are, it seems, some grounds of complaint against medical officers in other and, to my mind, more serious connections than those yet dealt with. It has occurred that the Medical Officer of Health of a district has acted as though he were the superior of the Public Analyst, in the sense of possessing a right to deal with the reports of the latter, publishing them as though they emanated from a mere departmental officer under his control. Such a distortion of fair conduct, or anything analogous to it, must be and remain rare, and need not be discussed as though it pertained to the normal. It could arise only from the existence of a complete misunderstanding of the situation. I am sure that every reasonable medical officer of health, recognising that the analyst's appointment is a direct one and coequal with his own, would wholly repudiate such a course. In such a case it would be well to see that the facts came to the knowledge of the medical press, in which, I am sure, it would meet with right and effective comment.

Leaving for the moment any further reference to the difficulties which have arisen between doctor and analyst, let me now proceed to the more satisfactory task of indicating future developments which may tend to bring them together.

In pursuit of this side of my subject, I find that I may logically begin by referring to the Institute examination in pharmacology and therapeutics. When this was first established by the fiat of the Local Government Board, Sir Thomas Stevenson, the first examiner, very wisely established a standard which has since kept the range of the examination within certain narrow limits. The student has been expected to recognise by their naked-eye characteristics the various drugs of the British Pharmacopoeia, and to know roughly the practical uses of the more important drugs, and the doses, medicinal and fatal, of such drugs as are presumably toxic.

Such limitations were very necessary on the first establishment of the subject, and the present view of the Council, which, with certain modifications, should, I think, be respected by all wise examiners, is in favour of the

careful avoidance of any demand for knowledge within the proper province of the medical student.

At the moment, however, things have arrived at this pass: Either as a result of the growth of coaching, or because of present organised facilities for handling pharmaceutical preparations, not a single candidate now fails to be well versed in the recognition of drugs. It is most rare to find a single individual who cannot, with the aid of various "tips" and *memoria technica*, recognise with unfailing accuracy every typical drug which he may be shown. His knowledge of doses is equally accurate, for he comes provided with a short list which has been got by heart the night before, and can be again consulted at the eleventh hour. Indeed, on the somewhat rare occasions when it becomes necessary to refer a candidate, it is almost always, in my experience, because of weakness in the associated subject of microscopy, in the practical technique of which I have been surprised to find a chemical candidate inferior to the medical student of similar standing.

Now, as examiner, I certainly did not wish for more opportunities of referring candidates, nor did I desire to increase the amount of work the student has to do. In asking for a somewhat more extended syllabus, I had several considerations in view which I should like to submit to you.

In the first place, I found that the candidates come up for this examination with feelings of considerable discomfort. So far as the matter of previous papers set on the lines defined above can guide them, they succeed easily in making themselves letter perfect; but they feel that between the boards of a textbook of pharmacology and therapeutics there are many things beyond these, and though they understand they are not to be treated as medical students, they yet feel uncertain as to what exactly may be sprung upon them, to their no small distress. In circumstances such as these a syllabus which seems to extend the subject may really limit the amount of reading to be done.

Next, it appeared to me that if a given subject is studied under compulsion, those parts of it which stimulate interest and are of educational value should certainly not be eliminated, and it seems to me that "spotting" drugs and learning doses by heart possess little of either of these qualities.

Again, there seemed to be aspects of pharmacology which really concern the professional chemist as much, if not more than, those hitherto emphasised. I have found, for instance, that candidates who can without hesitation distinguish between caraway and dill, or rattle off a list of doses, did not know that a man taking chloral hydrate excretes a product which, by reducing Fehling's solution, has led to confusion with glycosuria. Yet he might quite conceivably be face to face with the results of this phenomenon in his laboratory, and such matters, as well as many analogous ones, seemed to me to concern him even more than a knowledge of poisonous doses. Moreover, if the fatal dose of a poisonous drug is supposed to be known, there should certainly be the added knowledge of the influence of age, idiosyncrasy, and habituation as modifying such doses.

Questions as to whether a poisonous drug is rapidly eliminated, or whether it is accumulated in the body, and whether, when it has to be looked for, it will be found unaltered or changed by the extraordinarily interesting processes to which the body may submit it, are of import to the professional chemist; for, though it is to be hoped that investigations concerning criminal toxicology may largely remain in the hands of specialists, yet the help of the professional chemist in ordinary practice may often be sought in non-criminal cases. His help may, indeed, become more important, and at the same time his task somewhat more difficult, with the growing use of complex organic drugs.

It further seemed to me, though I was not allowed by the Council to insert such matters in the syllabus, that a chemical student who is compelled to learn pharmacology at all might well have his attention called to the interesting relationships which exist between the chemical constitution of active substances and their effect upon the body. This knowledge, and the synthetic work based upon it, has certainly proved highly profitable to scientific chemists in Germany.

Such knowledge as is asked for in the present syllabus can be got from almost any reasonably complete textbook by selecting a few special sections. It is not its bulk, but its suggestiveness, that gives it importance.

Note that it has not hitherto been my purpose to discuss the wisdom of the actual institution of a professional examination in pharmacology and therapeutics; but, taking it as an accomplished fact, I have wished to point out that, without taxing the student's time overmuch, and without treating him in any sense as a medical student, he may with great advantage be asked to know something more than the dry bones of the subject.

But I believe further that, just as the establishment of Branch F (biological chemistry) came at an opportune moment, and will help to provide experts whose services will be of great value to the State in the immediate future, so the existence of a section in Branch E capable of giving some slight medical bias to the minds of even a few students will ultimately prove of no small service, both to themselves and to the medical profession. This is my firm faith, and this is why I have wished to see the examination deal with something more than the dead and unstimulating aspects of the subject. I will try to explain why I hold this faith in the value of a medical bias just now.

As many of you will have realised, there has been enormously accentuated progress in physiological chemistry during quite recent years. The new knowledge being gained is living stuff and of real practical importance. Now, progress in physiology rapidly reacts upon pathology, and pathology upon methods of diagnosis. Pathology is striking out some chemical paths of its own, but it has not yet felt the effect of the unloading of chemical facts which physiology is preparing for it. Sooner or later there will be activity on lines unknown to the physician at present.

If we consider the question of urine analysis alone, it is easy to prognosticate that there will be added to the study of the few constituents which are now treated almost as that of a great number of excretives, appearing in small amount, but of great importance as measures of departures from the normal metabolism. The practical urine analyst of the future will have to erect an extensive ground. Even now, with the physician only vaguely aware that the pathological chemistry of the part, which was too ill developed a subject to be of much use to him, is giving way to something more real — even now there is some awakening to the importance of the laboratory. On the Continent, at any rate, and especially in France, elaborate and very numerous analytical studies are continuously made by professional analysts, from which characteristic curves are constructed, there being supposed to give important indications as to departures from normal health. Though many physicians in France attach much importance to these curves and attribute to them even a prognostic value, I believe myself that much of this work is only pseudo-scientific; it is a little premature and undiscriminating, but it is, at any rate, exceedingly profitable to the analyst, the patient, if not the physician — cheerfully paying satisfactory fees.

In this country the medical profession is, at any rate, beginning to think more seriously of the professional laboratory as an aid to its work. This is evidenced by the astonishing success of certain medical investigation associates to run upon a business line. Only part of their work is chemical, however, and as the complexity of the chemical problems grows, the special difficulties of chemical technique will send the work more and more into the hands of individual experts.

The average medical man, while not yet aware of all that chemistry can do for him, is also, for reasons already indicated, not yet clear as to whom he should turn to for help in those chemical problems which he has, even at the present time, in mind. I have read recent elaborate medical monographs, involving extraordinarily futile chemical investigations, in which the author, himself innocent of chemistry, acknowledges his obligations to the pharmacy which is adjacent to him. Now, it is no reflection upon an honourable calling to suggest that in such a case the doctor ought to have gone elsewhere. One can see that in these conjoint researches it was the good nature of the pharmacist, and no worse motive, which made him content to the unprofitable partnership. But I believe it is not going too far to say that the rise of chemical pathology to its full importance will call almost for a new profession.

I wish very much to make a point here which, if it seems too remote at present from your practical interests, may be considered as in parentheses. The care of the body in sickness, with all the delicacies of human relationship which it involves, must remain always an entire and carefully guarded prerogative of the physician; but the innate respect of the public, and even of the non-medical scientific public, for the physician's calling has led to a somewhat illogical attitude, and has tended to make sacrosanct not only

the calling of the physician, but the scientific material which he deals with. There has been, as it were, an averting of the gaze whenever a region of knowledge is stamped as "medical." Now, I am certain that the progress of scientific medicine demands a change here. While a large part of future scientific medical studies must always be carried out by men who, though medically qualified, have preferred the laboratory to practice, and whose special qualification, therefore, is that they have had personal touch with the problems offered by disease, yet in a middle region these must be joined in their work by men whose primary qualifications are non-medical—men who, saved from the long years of clinical study, are able to bring well-grounded laboratory knowledge and (I may add) a sufficient knowledge of the literature of pathology, which is open to all, to join their medically qualified confrères in attacking the huge problems which await solution.

Many of these must be organic chemists, and, to make a distinction which I will urge again later, many of them must be also analysts. If it be felt that these are matters which concern those who aim at pure science, and not the professional chemist, it is yet certain that the amount of valuable analytical work to be done professionally and outside the research laboratory will greatly increase as a result of the tendencies indicated.

My own calling compels me to know well the present position of physiological and pathological chemistry, and I may claim to have had first-hand acquaintance with the large amount of paying work which is even now being done for the medical profession, though not by individual professional analysts. And though I may not stop to prove my point further, I yet reiterate my opinion that there will be in the near future an efficient source of income for many of the students in your laboratories if they are prepared to work on these lines.

While upon the business of prophecy, I am tempted to put another series of prognostications before you, the credibility of which is at the present time, perhaps, more obvious to the physiological chemist than to anybody else. I pass from pathology to an aspect of dietetics. This is a subject in which the medical man is the recognised authority, charged with instruction of the public, but for a scientific knowledge of which he depends largely on the chemical physiologist and the analyst.

Putting on one side the aspect of affairs which especially concerns this Society—the maintenance of purity and freedom from adulteration—and leaving out questions such as digestibility and the like, the chief practical points which have hitherto been considered in relation to the daily rations of mankind are the total energy value requisite for maintenance, the optimum ratio of fats and carbohydrates, and the optimum supply of protein. Now, these questions have recently received fresh attention, and experimental work has been done lately yielding, as you know, somewhat startling results, tending at first sight to modify our views concerning maximal, minimal, and optimum dietaries. But I am not going to discuss the work of Atwater or Chittenden, proposing rather to put before you very briefly

facts of another sort, less known and seemingly academic. I believe, however, that my theme, which is that of the influence of minimal qualitative variations in dietaries, will one day become recognised as of great practical importance.

Physiological chemistry, chiefly owing to the work of Emil Fischer, has recently gained the knowledge that individual proteins, and among them those which contribute to human dietaries, may each bear a special chemical stamp; that a given protein may differ so widely from another protein as to have, quite possibly, a different nutritive value. I will illustrate this, first of all, by a somewhat extreme case. A protein, zein, forming no inconsiderable proportion of the total nitrogenous constituents of maize, is entirely deficient in at least one characteristic molecular grouping. It yields on digestion no tryptophane, the product which represents the indol group present in the molecules of most typical proteins.

In mentioning tryptophane, I cannot deny myself a moment's harmless gibe at your expense. The well-known colour reaction which you have used for so many years as a test for formaldehyde in milk is really a reaction due to this indol group of the casein. Now, as it was a similar colour reaction which led some of us at Cambridge to separate the tryptophane of protein for the first time, I have felt that some of you, being authorities on foodstuffs, ought with proper enterprise to have anticipated us in this not unimportant discovery.

Recently we have fed animals with this indol-free maize protein in such a way that it formed the only supply of protein, though associated with abundant fat and carbohydrate and suitable salts. The diet wholly failed to maintain tissue growth in young animals, which, however, grew at once when their zein was replaced by pure casein. When tryptophane was added to the zein diet, there was still inability to maintain tissue growth, doubtless because the zein has other deficiencies as a protein. But now an interesting fact came to light. The animals which received the missing indol derivative in addition to the zein did not grow, in fact, they continued to lose weight daily, but were afterwards in much better health than, and long outlived, those which had the zein alone. These experiments seem to show two important facts: First, that in an extreme case a particular protein may wholly fail to support life, just as is the case with gelatin; and next, that a group in the protein molecule may serve some purpose in the body other than that of forming tissue or supplying energy. The usual discussions about foodstuffs attribute to them these two functions only—repair of the tissues and energy supply. But the body has other and more subtle needs equally urgent. Here, there, or elsewhere in the organs must appear special, indispensable, active substances which the tissues can only make from special precursors in the diet.

The indol grouping in the protein molecule serves some such special purpose, quite distinct from its necessary function in tissue repair. This matter of qualitative differences in proteins may be of no small significance

in dietaries. It may account for what I believe is proved by experience—that rice may serve the races which rely upon it as an almost exclusive source of protein, while wheat is only suitable for races that take a much more varied dietary. It may explain many variations in nutritive values which at present we feel and recognise only vaguely. In the future the analyst will be asked to do more than determine the total protein of a foodstuff; he must essay the more difficult task of a discriminative analysis.

But, further, no animal can live upon a mixture of pure protein, fat, and carbohydrate, and even when the necessary inorganic material is carefully supplied the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or the tissues of other animals, and these contain countless substances other than the proteins, carbohydrates, and fats.

Physiological evolution, I believe, has made some of these well-nigh as essential as are the basal constituents of diet. Lecithin, for instance, has been repeatedly shown to have a marked influence upon nutrition, and this just happens to be something already familiar, and a substance that happens to have been tried. The field is almost unexplored; only is it certain that there are many minor factors in all diets of which the body takes account.

In diseases such as rickets, and particularly in scurvy, we have had for long years knowledge of a dietetic factor; but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure. They are, however, certainly of the kind which comprises these minimal qualitative factors that I am considering.

Scurvy and rickets are conditions so severe that they force themselves upon our attention; but many other nutritive errors affect the health of individuals to a degree most important to themselves, and some of them depend upon unsuspected dietetic factors.

I can do no more than hint at these matters, but I can assert that later developments of the science of dietetics will deal with factors highly complex and at present unknown.

But am I at present justified in troubling you, as practical men, with such matters—you who are interested in professional chemistry, and not in what is still more or less academic physiology?

I have been led to do so from two considerations. First, it is abundantly clear that the foundation of future progress in chemical pathology and dietetics on the lines I have been indicating calls for large efforts in purely analytical chemistry—efforts which have been too long delayed. And the delay has arisen from a circumstance of no small interest and importance.

The scientific chemist—unlike his predecessors, the pioneers of sixty or seventy years ago—has long ceased to be much interested in the animal or the plant. Further, the triumph of synthetic work in advancing theory has led the pure chemist away from the especial difficulties of analytical work. His extraordinarily developed technique concerns itself only secondarily and imperfectly with analytical studies of the kind still necessary in physiological problems. I mean the endeavours to identify and separate



1908.

"I can do no more than hint at these unsuspected dietetic factors, but I can assert that later developments of the science of dietetics will deal with factors highly complex and at present unknown. . . ." (p. 134).



unknown substance with unknown properties, present in complex mixtures. Only now and again has he made special efforts in this direction, such as that with which Fischer started his work upon proteins. Such work really requires special instincts, and the pure chemist has largely lost them. He is but a poor analyst, as the physiological explorer finds on turning to him for help. I feel that this help, so far as the immediate future is concerned, will have to come from the pupils primarily trained in your own laboratories, where the analytical instinct is developed. Some of your students, it is to be hoped, will have their attention turned in this direction, and to at least a few there may ultimately come opportunities for research; for research, in all callings, even that of the academic teacher, is only to be snatched from leisure. There are the beginnings just now of a renewed interest in biology on the part of all chemists. May the analyst feel this too. It is not only the manufacturer and the sanitary authority that require his help.

In the second place, I am not afraid to assert that progress in dietetics, no less than in chemical pathology, is about to react largely on professional chemical practice. Fresh problems and new ideas will unfailingly extend the field of professional operations.

All progress of the kind I have been hinting at cannot fail to be of the greatest importance to the doctor; and if I may seem to have maligned him in previous paragraphs, I know well how ready and able he is to make use of all knowledge that he believes to yield advantage to his patients.

I see abundant reasons for believing that in the near future events will march to the consummation of mutual appreciation and helpfulness, and to the disappearance of all misunderstanding, in the relations between analyst and medical man.

*Note.*—It is interesting and instructive to refer to the printed record of the discussion which followed this address: it shows how unprepared the participants and other listeners were to take up the new elements in Hopkins' thought. (Editors.)

## THE DYNAMIC SIDE OF BIOCHEMISTRY

[*Rep. Brit. Ass.* 1913, p. 652]

IN the year 1837 Justus Liebig, whom we may rightly name the father of modern animal chemistry, presented a Report to the Chemical Section of the British Association, then assembled at Liverpool. The technical side of this report dealt with the products of the decomposition of uric acid, with which I am not at the moment concerned, but it concluded with remarks which, to judge from other contemporary writings of Liebig, would have been more emphatic had the nature of his brief communication permitted. Liebig had a profound belief that in the then new science of Organic Chemistry, Biology was to find its greatest aid to progress, and his enthusiastic mind was fretted by the cooler attitude of others. In the report I have mentioned he called upon the chemists of this country to take note of what was in the wind, and while complimenting British physiologists and biologists upon their own work, urged upon them the immediate need of combining with the chemists. Ten years later, Liebig had still to write with reference to chemical studies: "Der Mann welcher in der Thierphysiologie wie Saussure in der Pflanzenphysiologie die ersten und wichtigsten Fragen zur Aufgabe seines Lebens macht, fehlt noch in dieser Wissenschaft."\* Much later still, he was making the same complaint. As a matter of fact, the combination of chemistry with biology, in the full and abundant sense which Liebig's earlier enthusiasm had pictured as so desirable, never happened in any country within the limits of his own century, while in this country, up to the end of that century, it can hardly be said to have happened at all. But the regrettable divorce between these two aspects of science has been so often dwelt upon that you will feel no wish to hear it treated historically, and perhaps even any emphasis given to it now may seem out of place, since on the Continent, and notably in America, the subject of Biochemistry (with its new and not very attractive name) has come with great suddenness into its kingdom. Even in this country, the recent successful formation of a Biochemical Society gives sure evidence of a greatly increased interest in this borderland of science. Yet I am going to ask you to listen to some remarks which are a reiteration of Liebig's appeal, as heard by this Association three quarters of a century ago.

For one can, I think, honestly say that it is yet a rare thing in this country to meet a professed biologist, even among those unburdened either with years or traditions, who has taken the trouble so to equip himself in organic chemistry as to understand fully an important fact of metabolism stated in terms of structural formulae. The new science of Physical Chemistry has

\* *Ann. d. Chem. u. Pharm.*, 1847, **62**, 257.

made a more direct appeal to the biological mind. Its results are expressed in more general terms and the bearing of its applications is perhaps more obvious, especially at the present moment. This fact increases the danger of a further neglect in biology of the organic structural side of chemistry, upon which, nevertheless, the whole modern science of intermediary metabolism depends. On the other hand, I think one may say that there are only a few among the present leaders of chemical thought in our midst who have set themselves to appraise with sympathy the drift of biological processes or the nature of the problems that biologists have before them. Anyone wishing to see the number of biochemical workers increased might therefore with equal justice appeal to the teachers of biology or to the teachers of chemistry for greater sympathy with the borderland. It is a moot point indeed as to which is the better side for that borderland to recruit its workers from.

But on the whole it is easier for the intelligent adult mind to grasp new problems than to learn a new technique. It is better that youth should be spent in acquiring the latter. That is why, though I admit that it would have been more obviously to the point if made some ten years ago, I feel justified in repeating to-day the appeal of Liebig to the leading chemists of this country, in the hope that they may see their way to direct the steps of more of their able students into the path of Biochemistry. I have been specially tempted to do this, rather than to speak upon some of many subjects which would have interested this section\* more, for a very practical reason. I have been in a position to review the current demand of various institutions, home and colonial, for the services of trained biochemists, and can say, I think with authority, that the demand will rapidly prove to be in excess of the supply. It will be a pity if the generation of trained chemists now growing up in this country should not share in the restoration of this balance. You certainly have the right to tell me that I ought, under the circumstances, to be addressing another section; but it may be long before any member of my cloth will have the opportunity of appealing to that section from the position of advantage that I occupy here. I believe you will forgive the particular trajectory of my remarks because I am sure you will sympathise with their aim. Moreover, I have some hope that the considerations upon which I shall chiefly base my appeal will have some interest for members of this section as well as for the chemist. My main thesis will be that *in the study of the intermediate processes of metabolism we have to deal, not with complex substances which elude ordinary chemical methods, but with simple substances undergoing comprehensible reactions*. By simple substances I mean such as are of easily ascertainable structure and of a molecular weight within a range to which the organic chemist is well accustomed. I intend also to emphasise the fact that it is *not alone with the separation and identification of products from the animal that our present studies deal; but with their reactions in the body; with the dynamic side of biochemical phenomena*.

\* Physiology.

I have made it my business during the last year or two to learn, by means of indirect and most diplomatic inquiries, the views held by a number of our leading organic chemists with respect to the claims of animal chemistry. I do not find any more the rather pitying patronage for an inferior discipline, and certainly not that actual antagonism, which fretted my own youth; but I do find still very widely spread a distrust of the present methods of the Biochemist, a belief that much of the work done by him is amateurish and inexact. What is much more important, and what one should be much more concerned to deny (though but a very small modicum of truth is, or ever was, in the above indictment), is the view that such faults are due to something inherent in the subject.

My desire is to point out that continuous progress, yielding facts which, by whomsoever appraised, belong to exact science, has gone on in the domain of animal chemistry from the days of Liebig until now, and that if this progress was till recently slow, that was, in the main, due to a continuation of the circumstance which so troubled Liebig himself—the shortage of workers.

But we must also remember that the small band of investigators who concerned themselves with the chemistry of the animal in the latter half of the nineteenth century suffered very obviously from the fact that the channels in which chemistry as a whole was fated to progress left high and dry certain regions of the utmost importance to their subject. In three regions particularly the needs of Biochemistry were insistent. The colloid state of matter dominates the milieu in which vital processes progress, but, notwithstanding the stimulating work of Graham, the pure chemist of the last century consistently left colloids on one side with a shudder of distaste. Again, we have come to recognise that the insidious influence of catalysts is responsible for all chemical change as it occurs in living matter, but for many years after Berzelius the organic chemist gave to the subject of catalysis very cursory attention, fundamental though it be. Lastly, every physiological chemist has to realise that among his basal needs is that of accurate methods for the estimation of organic substances when they are present in complex mixtures. But the organic chemist of the nineteenth century did not develop the art of analysis on these lines. Of the myriad substances, natural or artificial, known to him, at the most a few score could be separated quantitatively from mixtures, or estimated with any accuracy. It was a professional or commercial call rather than scientific need which evolved such processes as were available, so that this side of chemical activity developed only on limited and special lines.

All these circumstances were, of course, inevitable. Organic chemistry in Liebig's later years was concerned with laying its own foundations as a pure science, and for the rest of the century with building a giant, self-contained edifice upon them. The great business of developing the concepts of molecular structure and the wonderful art of synthesis were so absorbing as to leave neither leisure nor inclination for extraneous

labours. But it is easy to recognise that, near the beginning of the present century, a sense of satiety had arisen in connection with synthetic studies carried out for their own sake. Workers came to feel that, so far as the fundamental theoretical aspects of chemistry were concerned, that particular side of organic work had played its part. In numerous centres, instead of only in a few, quite other aspects of the science were taken up: in particular, the study of the dynamic side of its phenomena. The historian will come to recognise that a considerable revolution in the chemical mind coincided roughly with the beginning of this century. Among the branches which are fated to benefit by this revolution—it is to be hoped in this country as well as others—is the chemistry of the animal.

But I would like to say that I do not find, on reading the contributions to science of those who, as professed physiological chemists, ploughed lonely furrows in the last century, any justification for the belief that the work done by them was amateurish or inexact; no suggestion that anything inherent in the subject is prone to lead to faults of the kind. Truly these workers had to share ignorance which was universal, and sometimes, compelled by the urgency of certain problems, had perforce to do their best in regions that were dark. But they knew their limitations here as well as their critics did, and relied for their justification upon the application of their results, which was often not understood at all by their critics.

There is little doubt, for instance, that it was the earlier attempts of various workers to fractionate complex colloid mixtures which led to the cynical statement "*Thierchemie* is *Schmierchemie*." But the work thus done, even such work as Kühne's upon the albumoses and peptones, had important bearings, and led indirectly to the acquirement of facts of great importance to physiology and pathology.

In connection with enzyme catalysis the work done at this time by physiological chemists was in the main of a pioneer character, but it was urgently called for and had most useful applications. By the end of the century, indeed, it had become of great importance. I recall an incident which illustrates the need of suspended judgment before work done in new regions is assumed to be inexact. In 1885 E. Schütz published a study of the hydrolysis of protein by pepsin which showed that the rate of action of the ferment is proportionate to the square root of its concentration. When this paper was dealt with in Maly's *Jahresbericht* the abstractor (who from internal evidence I believe was Richard Maly himself) believed so little in such an apparent departure from the laws of mass action that he saw fit to deal with the paper in a ribald spirit and to add, as a footnote to his abstract, the lines:

"Musst mir meine Erde  
Doch lassen steh'n  
Und meine Hütte die du nicht gebaut!"

Yet it is now known that the relation brought to light by Schütz does hold for certain relative concentrations of ferment and substrate. That it

had limitations was shown by Schütz himself. The fact, however, involves no such shaking of the foundations as the abstractor thought. We quite understand now how such relations may obtain in enzyme-substrate systems.

As for analytical work involving a separation of complex organic mixtures the biochemist of the last century was in this ahead of the pure organic chemist, as the development of urinary analysis if considered alone will show.

In countless directions the acquirement of exact knowledge concerning animal chemistry has been, as I have already claimed, continuous from Liebig's days till now. I would like in a brief way to illustrate this, and if I choose for the purpose one aspect of things rather than another, it is because it will help me in a later discussion. I propose to remind you of certain of the steps by which we acquired knowledge concerning the synthetic powers of the animal body, apologising for the great familiarity of many of the facts which I shall put before you.

It seems that the well-known Glasgow chemist and physician, Andrew Ure, was the first actually to prove, from observations made upon a patient, that an increased excretion of hippuric acid follows upon the administration of benzoic acid. Wöhler had earlier fed a dog upon the latter substance, and decided at the time that it was excreted unchanged; but when, later, Liebig had made clear the distinction between the two acids, Wöhler recalled the properties of the substance excreted by his dog, and decided that it must have been hippuric acid and not benzoic acid itself. Excited by the novel idea that a substance thus extraneously introduced might be caught up in the machinery of metabolism, Wöhler, immediately after the publication of Dr. Ure's statement, initiated fresh experiments in his laboratory at Göttingen, where Keller, by observations made upon himself, showed unequivocally that benzoic acid is, and can be on a large scale, converted into hippuric acid in the body. Thus was established a fact which is now among the most familiar, but which at that time stirred the imagination of chemists and physiologists not a little. The discovery immediately led to a large number of observations dealing with various conditions which affect the synthesis, but we may pass to the acute observations of Bertagnini. This investigator wished to earmark, as it were, the benzoic acid administered to the animal, in order to make sure that it was the same molecule which reappeared in combination. He so marked it with a nitro-group, giving nitro-benzoic acid and observing the excretion of nitro-hippuric acid. Later on he continued this interesting line of research by giving other substituted benzoic acids, and showed that in each case a corresponding substituted hippuric acid was formed. Even so far back as the earlier 'fifties a clear understanding was thus established that the body was possessed of a special mechanism capable of bringing a particular class of substances into contact with the amino-acid glycine, and of converting them, by means of a synthetic condensation (which had not then been induced by any laboratory method), into conjugates which, as later experiments have shown, are invariably less noxious for the tissues than the substances introduced.

Great is the number of compounds which are now known to suffer this fate. To the story begun by Ure and Wöhler, chapter after chapter has been added continuously up to the present day. In 1876 came the classical experiments of Bunge and Schmiedeberg. After laborious but successful efforts to obtain a good method for the estimation of hippuric acid in animal fluids, these authors proved, by a method of exclusion, that, in the dog at least, the kidney is the seat of the hippuric synthesis. When, in their carefully controlled experiments, blood containing benzoic acid and glycine was circulated through that organ, after its isolation from the body, the production of hippuric acid followed. Schmiedeberg, a little later, convinced himself that the reaction in the kidney was a balanced one; the organ can not only synthesise hippuric acid, it can also hydrolyse it. As with reactions elsewhere, so in the kidney cell, the equilibrium of the reaction depends on the relative concentration of the products concerned. Schmiedeberg then separated from the tissues of the kidney what he believed to be an enzyme capable of inducing the hydrolysis. Mutch, with improved methods, has recently shown that a preparation from the kidney, wholly free from intact cells, can, beyond all doubt, hydrolyse hippuric acid under rigidly aseptic conditions, the reaction being one which comes to an equilibrium point when some 97 per cent. of the substance is broken down. The occurrence of this equilibrium, and the form of the reaction-velocity curve as obtained by Mutch, suggested that synthesis under the influence of the enzyme was to be expected, and, on submitting the mixture of benzoic and glycine to its influence, Mutch obtained a product which, though too small in amount for analysis, was almost certainly hippuric acid. I have myself obtained evidence which shows that the synthesis does certainly occur under these conditions.

The significance of this earliest known synthesis in the body is no limited one. The amide linkage established by it is one with which the body deals widely, and is, of course, of the type which is dominant in tissue complexes, since it is one which unites the amino-acids in the protein molecule.

Seeing, from the nature of the material supplied for the synthesis by the body itself, that the foreign substances administered must intrude themselves into the machinery of protein metabolism, it is not surprising that many have turned their minds to consider how far a detailed study of the phenomena might throw light upon this machinery. How far can the body extend its supply of glycine when stimulated by increasing doses of benzoic acid? What effects follow when administration is pushed to its limits? How is the fate in metabolism of the whole molecule of protein affected when one particular amino-acid is inharmoniously removed? Can the amino-acid be itself synthesised *de novo* in response to the call for it? These and similar questions clearly arise. I can only stop to remind you that there is evidence that, in connection with this particular chemical synthesis, the carnivore reacts differently to the herbivore. If the body of the former be flooded with benzoic acid, only a proportion undergoes condensation. Only so

much glycine is supplied as would correspond, roughly, at any rate, with that rendered available by the normal contemporary breakdown of protein, whereas, in the herbivorous animal, pushing the administration of benzoic acid may lead to the excretion of so much conjugated glycine that it may contain more than half of the whole nitrogen excreted. This is, of course, much more than could come from the protein of the body, and it would seem that the amino-acid is prepared *de novo* for an express purpose, a significant thing. But I must not stop to consider questions which are still in course of study. Before the hippuric synthesis was first observed synthetic powers were thought to be absent from the animal. Since then we have been continuously learning of fresh instances of synthesis in the body, not only in connection with its treatment of foreign substances, with which I am just now concerned, but in connection with all its normal processes.

Another most interesting group of syntheses in which substances are so dealt with in the body as to reappear in conjugation with protein derivatives are those in which the sulphur group plays its part. In 1876 Baumann first introduced us to the ethereal sulphates of the urine, and, from much subsequent work, we know how great a group of substances, chiefly those of phenolic character, are, after administration, excreted linked to sulphuric acid. We have evidence to show that, in all probability, the original condensation is not with sulphuric acid itself, but that oxidation of a previously formed sulphur-containing conjugate has preceded excretion, and we know that another group of substances leave the body combined with unoxidised sulphur. Certain cyanides—the aliphatic nitriles, for example—reappear as sulpho-cyanides; but above all in interest is the case described by Baumann, in which the intact cystein complex of protein, after suffering acetylation of its amino group, is excreted as a conjugate. The administration of halogen-benzene compounds is followed by the appearance of the so-called mercapturic acids in which the cystein is linked by its sulphur atom to the ring of chlor-, bromo-, or iodo-benzene. That large amounts of these conjugates can be formed during the twenty-four hours is certain, but it would be interesting to know what limit is set to this loss of cystein from the body.

I will now recall to you syntheses in which the substance supplied by the body is derived, not from protein, but from carbohydrate. The study of the fate of camphor in the body, carried out by Schmiedeberg and Hans Meyer in 1878, if it stood by itself, would abundantly illustrate the significance of this type of experiment. As you are aware, these workers proved that, after the administration of camphor, the urine contains a conjugate formed between an oxidation product of the camphor and an oxidation product of glucose. Both substances were then new to chemistry, and the latter—glycuronic acid—has since proved itself of great physiological interest. After Schmiedeberg's and Hans Meyer's experiments it was realised for the first time that the sugar molecule might play a part in metabolism quite distinct from its function as fuel, a fact that has much of

agency at the present time. We have good reason to believe that though, as a matter of fact, glycuronic acid is a normal metabolite, the actual synthesis concerns sugar itself, the oxidation of the glucose molecule occurring later. The compound formed is of the glucoside type, and the analogy with the formation of glucosides in the plant is unmistakable. Already the number of substances known to suffer this particular synthesis is legion. Almost every organic group yields an example.

Lastly, in illustration of a quite different type of synthesis (I can only deal with a few of the many known cases) we may recall the methylation which certain compounds undergo. The mechanism of this process, as it occurs in the body, is obscure, and its explanation would be of the greatest chemical interest. I must mention only one particular instance investigated by Achermann. When nicotinic acid is fed to animals, it is excreted as trigonellin, a hitherto vegetable base. This conversion involves methylation, and is of striking character as an instance of the artificially induced production of a plant alkaloid in the animal body.

The full significance of all such happenings will not be understood unless it be remembered that a nice adjustment of molecular structure is in many cases necessary to prepare the foreign substance for synthesis. Preliminary regulated oxidations or reductions may occur so as to secure, for example, the production of an alcoholic or phenolic hydroxyl group, which then gives the opportunity for condensation which was otherwise absent.

I have touched only on the fringes of this domain. The body of knowledge available concerning it has not been won systematically, and the fate of a multitude of other types of organic substances remains for investigation. The known facts have, one feels, an academic character in the view of the physiologist, and even in that of the pharmacologist, to whom we owe most of our knowledge about them. But, in my opinion, the chemical response of the tissues to the chemical stimulus of foreign substances of simple constitution is of profound biological significance. Apart from its biological bearings as the simplest type of immunity reaction, it throws vivid light, and its further study must throw fresh light, on the potentialities of the tissue laboratories.

In a brilliant address delivered before the Faculty of Medicine of the University of Leeds, Lord Moulton likened the process of recovery in the tissues after bacterial invasion to the generation of forces which establish what is known to the naval architect as the "righting couple." This grows greater the greater the displacement of a ship, and finally may become sufficient to overpower the forces tending to make her heel over. It is surely striking to realise that the establishment of the "righting couple" which brings the tissue cell back to equilibrium after the disturbances due to the intrusion of simple molecules calls for such a complex of chemical events, events which ultimately result in the modification of the disturbing substance and its extrusion from the tissues concerned in a form less noxious to the body as a whole.

Oxidation, reduction, desaturation, alkylation, acylation, condensation; any or all of these processes may be brought *de novo* into play as the result of the intrusion of a new molecule into reactions which were in dynamic equilibrium. It is clear that chemical systems capable of so responding to what may be termed specific chemical stimuli must not be neglected by any student of chemical dynamics. The physiologist has for many years been engaged upon careful analyses of the mechanical and electric responses to stimulation. In the phenomena before us we find "responses" which are equally fundamental. If we do not study them exhaustively we shall miss an important opportunity for throwing light upon the nature of animal tissues as chemical systems.

One reason which has led the organic chemist to avert his mind from the problems of Biochemistry is the obsession that the really significant happenings in the animal body are concerned in the main with substances of such high molecular weight and consequent vagueness of molecular structure as to make their reactions impossible of study by his available and accurate methods. There remains, I find, pretty widely spread, the feeling—due to earlier biological teaching—that, apart from substances which are obviously excreta, all the simpler products which can be found in cells or tissues are as a class mere dejecta, already too remote from the fundamental biochemical events to have much significance. So far from this being the case, recent progress points in the clearest way to the fact that the molecules with which a most important and significant part of the chemical dynamics of living tissues is concerned are of a comparatively simple character. The synthetic reactions which we have already considered surely prepare us for this view; but it may be felt that, however important, they represent abnormal events, while the study of them has been largely confined to determining the end-products of change. Let me now turn to normal metabolic processes and to intermediary reactions.

We know first of all that the raw material of metabolism is so prepared as to secure that it shall be in the form of substances of small molecular weight; that the chief significance of digestion, indeed, lies in the fact that it protects the body from complexes foreign to itself. Abderhalden has ably summarised the evidence for this and has shown us also that, so far as the known constituents of our dietaries are concerned, the body is able to maintain itself when these are supplied to it wholly broken down into simple *Bausteine*, any one of which could be artificially synthesised with the aid of our present knowledge. Dealing especially with the proteins, we have good reason to believe that the individual constituent amino-acids, and not elaborate complexes of these, leave the digestive tract, while Folin, van Slyke, and Abel have recently supplied us with suggestive evidence for the fact that the individual amino-acids reach the tissues as such and there undergo change. But still more important, when things are viewed from my present standpoint, is the fact that recent work gives clear promise that we shall ultimately be able to follow, on definite chemical lines, the

fate in metabolism of each amino-acid individually; to trace each phase in the series of reactions which are concerned in the gradual breakdown and oxidation of the molecule. Apart from the success to which it has already attained, the mere fact that the effort to do this has been made is significant. To those at least who are familiar with the average physiological thought of thirty years ago, it will appear significant enough. So long as there were any remains of the instinctive belief that the carbonic acid and urea which leave the body originate from oxidations occurring wholly in the vague complex of protoplasm, or at least that any intermediate products between the complex and the final excreta could only be looked for in the few substances that accumulate in considerable amount in the tissues (for instance, the creatin of muscle), the idea of seriously trying to trace within the body a series of processes which *begin* with such simple substances as tyrosin or leucin was as foreign to thought as was any conception that such processes could be of fundamental importance in metabolism. However vaguely held, such beliefs lasted long after there was justification for them; their belated survival was due, it seems to me, to a certain laziness exhibited by physiological thought when it trenched on matters chemical; they disappeared only when those accustomed to think in terms of molecular structure turned their attention to the subject. But it should be clearly understood that the progress made in these matters could only have come through the work and thought of those who combined with chemical knowledge trained instinct and feeling for biological possibilities. Our present knowledge of the fate of amino-acids, as of that of other substances in the body, has only been arrived at by the combination of many ingenious methods of study. It is easy in the animal, as in the laboratory, to determine the end-products of change; but, when the end result is reached in stages, it is by no means easy to determine what are the stages, since the intermediate products may elude us. And yet the whole significance of the processes concerned is to be sought in the succession of these stages. In animal experiments directed to the end under consideration, investigators have relied first of all upon the fact that the body, though the seat of a myriad reactions and capable perhaps of learning, to a limited extent and under stress of circumstances, new chemical accomplishments, is in general able to deal only with what is customary to it. This circumstance has yielded two methods of determining the nature of intermediate products in metabolism. Considerations of molecular structure will, for instance, suggest several possible lines along which a given physiological substance may be expected to undergo change. We may test these possibilities by administering various derivatives of the substance in question. Only those which prove on experiment to be fully metabolised, or to yield derivatives in the body identical with those yielded by the parent substance, can be the normal intermediate products of its metabolism. All others may be rejected as not physiological. In a second method dependent upon this eclecticism of the body, substances are administered which so far differ from the normal

that, instead of suffering a complete breakdown, they yield some residual derivative which can be identified in the excreta, and the nature of which will throw light upon the chemical mechanism which has produced it. For instance, a substance with a resistant (because abnormal) ring structure, but possessing a normal side chain, may be used to demonstrate how the side chain breaks down. Again, we may sometimes obtain useful information by administering a normal substance in excessive amounts, when certain intermediate products may appear in the excreta. Another most profitable method of experiment is that in which the substance to be studied is submitted to the influence of isolated organs instead of to that of the whole animal. Under these conditions, a series of normal reactions may go on, but with altered relative velocities, so that intermediate products accumulate; or again when, as may happen, the successive changes wrought upon a substance by metabolism occur in different organs of the body, this use of isolated organs enables us to dissect, as it were, the chain of events. Extraordinarily profitable have been the observations made upon individuals suffering from those errors of metabolism which Dr. Garrod calls "metabolic sports, the chemical analogues of structural malformations." In these individuals Nature has taken the first essential step in an experiment by omitting from their chemical structure a special catalyst which at one point in the procession of metabolic chemical events is essential to its continuance. At this point there is arrest, and intermediate products come to light.

As you know, most ingenious use of this ready-made experimental material has added greatly to our knowledge of intermediate metabolism. Admirable use, too, has been made of the somewhat similar conditions presented by diabetes, clinical and experimental. Every day our knowledge of the dynamics of the body grows upon these lines.

I know that the history of all these efforts is familiar to you, but I am concerned to advertise the fact that our problems call for ingenuity of a special sort, and to point out that an equipment in chemical technique alone would not have sufficed for the successful attack which has been made upon them. But I am even more concerned to point out that the direct method of attack has been too much neglected, or has been in the hands of too few; I mean the endeavour to separate from the tissues further examples of the simpler products of metabolic change, no matter how small the amount in which they may be present; an endeavour which ought not to stop at the separation and identification of such substances, but to continue till it has related each one of them to the dynamic series of reactions in which each one is surely playing a part. The earliest attempts at tracing the intermediate processes of metabolism looked for information to the products which accumulate in the tissues, but it seemed to be always tacitly assumed that only those few which are quantitatively prominent could be of importance to the main issues of metabolism. It is obvious, however, upon consideration, that the degree to which a substance accumulates is

by itself no measure of its metabolic importance; no proof as to whether it is on some main line of change, or a stage in a quantitatively unimportant chemical bypath. For, if one substance be changing into another through a series of intermediate products, then, as soon as dynamical equilibrium has been established in the series, and to such equilibrium tissue processes always tend, the rate of production of any one intermediate product must be equal to the rate at which it changes into the next, and so throughout the series. Else individual intermediate products would accumulate or disappear, and the equilibrium be upset. Now the rate of chemical change in a substance is the product of its efficient concentration and the velocity constant of the particular reaction it is undergoing. Thus the relative concentration of each intermediate substance sharing in the dynamic equilibrium, or, in other words, the amount in which we shall find it at any moment in the tissue, will be inversely proportional to the velocity of the reaction which alters it. But the successive velocity constants in a series of reactions may vary greatly, and the relative accumulation of the different intermediate products must vary in the same degree. It is certain that in the tissues very few of such products accumulate in any save very small amount, but the amount of a product found is only really of significance if we are concerned with any function\* which it may possibly possess. It is of no significance as a measure of the quantitative importance of the dynamical events which give rise to it.

To take an instance. The substance creatin has always asserted itself in our conceptions concerning nitrogenous metabolism because of the large amount in which it is found in the muscle. It may be of importance *per se*, and abnormalities in its fate are certainly important as an indication of abnormalities in metabolism, but we must remember that the work of Gulewitsch, Krimberg, Kutscher, and others has shown us that a great number of nitrogenous basic bodies exist in muscle in minute amounts. Maybe we shall need to know about each of these all that we now know, or are laboriously trying to know, about creatin, before the dynamics of basic nitrogen in muscle become clear. Fortunately, for the experimenter, most of the raw materials required for tissue analysis are easily obtainable; there is no reason save that of the labour involved why we should not work upon a ton of muscle or a ton of gland tissue.

I am certain that the search for tissue products of simple constitution has important rewards awaiting it in the future, so long as physiologists are alive to the dynamical significance of all of them. Such work is laborious and calls for special instincts in the choice of analytical method, but, as I mentioned in an earlier part of this address, I am sure that high qualifications as an analyst should be part of the equipment of a biological chemist.

I should like now to say a few words concerning the actual results of this

\* A product of metabolism can only be said to have a "function" in a cell or in the body when, being the end-product of one reaction, it initiates or modifies reactions in another milieu.

modern work upon intermediate metabolism, and will return to the amino-acids. It is clear that what I can say must be very brief.

We know that the first change suffered by an  $\alpha$ -amino-acid when it enters the metabolic laboratories is the loss of its amino group, and, thanks to the labours of Knoop, Neubauer, Embden, Dakin, and others, we have substantial information concerning the mechanism of this change. The process involved in the removal of the amino group is not a simple reduction, which would yield a fatty acid, or substituted fatty acid, nor a hydrolytic removal which would leave an  $\alpha$ -hydroxy-acid; but the much less to be expected process of an oxidative removal, which results in the production of a keto-acid.\* If the direct evidence for this chemically most interesting primary change were to be held insufficient (though there is no insufficiency about it), its physiological reality is strongly supported by the proof given us by Knoop and Embden that the liver can re-synthesise the original amino-acid from ammonia and the corresponding keto-acid. This profoundly significant observation is part of the evidence which is continually accumulating to show that all normal chemical processes of the body can suffer reversal. The next step in the breakdown involves the oxidation of the keto-acid, with the production of a fatty acid containing one carbon less than the original amino-acid. This in turn is oxidised to its final products along the lines of the  $\beta$ -oxidation of Knoop, two carbon atoms being removed at each stage of the breakdown. All this is true of the aliphatic  $\alpha$ -amino-acids, and, with limitations, of the side chains of their aromatic congeners. In the case of certain amino-acids the course of breakdown passes through the stage of aceto-acetic acid. This happens to those of which the molecule contains the benzene ring, and Dakin has enabled us to picture clearly the path of change which involves the opening of the ring. This particular stage does not seem to occur in the breakdown of the aliphatic amino-acids, save in the case of leucin; the rule and the exception here being alike easy of explanation by considerations of molecular structure.

But direct breakdown on the lines mentioned is far from being the only fate of individual amino-acids in the body. The work of Lusk, completed by that of Dakin, has shown us that of seventeen amino-acids derived from protein no less than nine may individually yield glucose in the diabetic organism, and there are excellent grounds for believing (indeed, there is no doubt) that they do the same to a duly regulated extent in the normal organism. The remaining seven have been shown not to yield sugar, and there is therefore a most interesting contrast in the fate of two groups of the protein *Bausteine*. Those which yield sugar do not yield aceto-acetic acid, and those which yield the latter are not glycogenic. One set, after

\* Dakin's recent work is giving us an insight into the mechanism of the keto-acid formation. Amino-acids in aqueous solution dissociate into ammonia and the corresponding keto-aldehyde. The oxidation involved is therefore concerned with the conversion of the aldehyde into the acid.

undergoing significant preliminary changes, seems to join the carbohydrate path of metabolism, the other set ultimately joins a penultimate stage in the path which is traversed by fats.

I will here venture to leave for one moment the firm ground of facts experimentally ascertained. Unexplored experimentally, but quite certain so far as their existence is concerned, are yet other metabolic paths of prime importance, along which individual amino-acids must travel and suffer change. We know now from the results of prolonged feeding experiments upon young growing animals, which I myself, as well as many others, have carried out, that all the nitrogenous tissue complexes, as well as the tissue proteins, can be duly constructed when the diet contains no other source of nitrogen besides the amino-acids of protein. The purin and pyrimidin bases, for instance, present in the nuclear material of cells certainly take origin from particular amino-acids, though we have no right to assume that groups derived from carbohydrates or fats play no part in the necessary syntheses. While recent years have given us a wonderfully clear picture as to how the nucleic acids and the purin bases contained in them break down during metabolism, we have as yet no knowledge of stages in their synthesis. But it is clear that to discover these is a task fully open to modern experimental methods, and though a difficult problem, it is one ready to hand. Again, in specialised organs substances are made which are of great importance, not to the structure, but to the dynamics of the body. These have become familiar to us under the name of Hormones. We know the constitution of one only of these, adrenaline. The molecule of this exemplar has a simple structure of a kind which makes it almost certain to be derived from one of the aromatic amino-acids. It is clearly open to us to discover on what lines it takes origin. Facts of this kind, we may be sure, will form a special chapter of biochemistry in the future. I would like to make a point here quite important to my main contention that metabolism deals with simple molecules. As a pure assumption it is often taught, explicitly or implicitly, that although the bowel prepares free amino-acids for metabolism, only those which are individually in excess of the contemporary needs of the body for protein are directly diverted to specialised paths of metabolism, and these to the paths of destructive change. All others—all those which are to play a part in the intimacies of metabolism—are supposed to be first reconstructed into protein, and must therefore again be liberated from a complex before entering upon their special paths of change. But there is much more reason (and some experimental grounds) for the belief that the special paths (of which only one leads to the repair or formation of tissue protein) may be entered upon straight-way. Mrs. Stanley Gardiner (then Miss Willcock) carried out some feeding experiments a few years ago, and in discussing these I pointed out that they offered evidence of the direct employment for special purposes of individual amino-acids derived as such from the bowel. It seemed at the time that the argument was misunderstood or felt to carry little weight,

but later Professor Kossel\* quoted my remarks with approval and expressed agreement with the view that the *Bausteine* of the food protein must, in certain cases, be used individually and directly.

I wish I had time to illustrate my theme by some of the abundant facts available from quite other departments of metabolism; but I must pass on.

The chief thing to realise is that as a result of modern research the conception of metabolism in block is, as Garrod puts it, giving place to that of metabolism in compartments. It is from the behaviour of simple molecules that we are learning our most significant lessons.

Now interest in the chemical events such as those we have been dealing with may still be damped by the feeling that, after all, when we go to the centre of things, to the bioplasm, where these processes are initiated and controlled, we shall find a milieu so complex that the happenings there, although they comprise the most significant links in the chain of events, must be wholly obscure, when viewed from the standpoint of structural organic chemistry. I would like you to consider how far this is necessarily the case.

The highly complex substances which form the most obvious part of the material of the living cell are relatively stable. Their special characters, and in particular the colloidal condition in which they exist, determine, of course, many of the most fundamental characteristics of the cell: its definite yet mobile structure, its mechanical qualities, including the contractility of the protoplasm, and those other colloidal characters which the modern physical chemist is studying so closely. For the dynamic chemical events which happen within the cell, these colloid complexes yield a special milieu, providing, as it were, special apparatus, and an organised laboratory. But in the cell itself, I believe, simple molecules undergo reactions of the kind we have been considering. These reactions, being catalysed by colloidal enzymes, do not occur in a strictly homogeneous medium, but they occur, I would argue, in the aqueous fluids of the cell under just such conditions of solution as obtain when they progress under the influence of enzymes *in vitro*.

There is, I know, a view which, if old, is in one modification or another still current in many quarters. This conceives of the unit of living matter as a definite, if very large and very labile molecule, and conceives of a mass of living matter as consisting of a congregation of such molecules in that definite sense in which a mass of, say, sugar is a congregation of molecules, all like to one another. In my opinion, such a view is as inhibitory to productive thought as it is lacking in basis. It matters little whether in this connection we speak of a "molecule" or, in order to avoid the fairly obvious misuse of a word, we use the term "biogen," or any similar expression with the same connotation. Especially, I believe, is such a view unfortunate when, as sometimes, it is made to carry the corollary that

\* *Johns Hopkins Hospital Bulletin*, March, 1912.

simple molecules, such as those provided by foodstuffs, only suffer change after they have become in a vague sense a part of such a giant molecule or complex. Such assumptions became unnecessary as soon as we learnt that a stable substance may exhibit instability after it enters the living cell, not because it loses its chemical identity, and the chemical properties inherent in its own molecular structure, by being built into an unstable complex, but because in the cell it meets with agents (the intracellular enzymes) which catalyse certain reactions of which its molecule is normally capable.

Exactly what sort of material might, in the course of cosmic evolution, have first come to exhibit the elementary characters of living stuff, a question raised in the Presidential Address which so stirred us last year, we do not, of course, know. But it is clear that the living cell as we now know it is not a mass of matter composed of a congregation of like molecules, but a highly differentiated system; the cell, in the modern phraseology of physical chemistry, is a system of co-existing phases of different constitutions.\* Corresponding to the difference in their constitution, different chemical events may go on contemporaneously in the different phases, though every change in any phase affects the chemical and physico-chemical equilibrium of the whole system. Among these phases are to be reckoned not only the differentiated parts of the karyoplasm strictly defined (if we can define it strictly) the macro- and micronuclei, nerve fibres, muscle fibres, etc., but the material which supports the cell structure, and what have been termed the "metaplasmic" constituents of the cell. These last comprise not only the fat droplets, glycogen, starch grains, aleurone grains, and the like, but other deposits not to be demonstrated histologically. They must be held, too, a point which has not been sufficiently insisted upon—to comprise the diverse substances of smaller molecular weight and greater solubility, which are present in the more fluid phases of the system—namely, in the cell juices. It is important to remember that changes in any one of these constituent phases, including the metaplasmic phases, must affect the equilibrium of the whole cell system, and because of this necessary equilibrium-relation it is difficult to say that any one of the constituent phases, such as we find *permanently* present in a living cell, even a metaplasmic phase, is less essential than any other to the "life" of the cell, at least when we view it from the standpoint of metabolism. It is extremely difficult and probably impossible by any treatment of the animal to deprive the liver completely of its glycogen deposits, so long as the liver cells remain alive. Even an extreme variation in the quantity is in the present connection without significance because, as we know, the equilibrium of a polyphasic system is independent of the mass of any one of the phases; but I am inclined to the bold statement that the integrity of metabolic life of a liver cell is as much dependent on the co-existence of metaplasmic glycogen,

\* See in this connection the very able exposition of the views developed by Zwaardemaker and others, by Botazzi in Winterstein's *Handbuch*, Vol. 1.

however small in amount, as upon the co-existence of the nuclear material itself; so in other cells, if not upon glycogen, at least upon other metaplasmic constituents.

Now we should refuse to speak of the membrane of a cell, or of its glycogen store, as living material. We should not apply the term to the substances dissolved in the cell juice, and, indeed, would hardly apply it to the highly differentiated parts of the bioplasm if we thought of each detail separately. We are probably no more justified in applying it, when we consider it by itself, to what, as the result of microscopic studies, we recognise as "undifferentiated" bioplasm. On ultimate analysis we can hardly speak at all of living matter in the cell; at any rate, we cannot, without gross misuse of terms, speak of the cell life as being associated with any one particular type of molecule. *Its life is the expression of a particular dynamic equilibrium which obtains in a polyphasic system.* Certain of the phases may be separated, mechanically or otherwise, as when we squeeze out the cell juices, and find that chemical processes still go on in them; but "life," as we instinctively define it, is a property of the cell as a whole, because it depends upon the organisation of processes, upon the equilibrium displayed by the totality of the co-existing phases.

I return to my main point. The view I wish to impress upon you is that some of the most important phenomena in the cell, those involving simple reactions of the type which we have been discussing, occur in ordinary crystalloid solution. We are entitled to distinguish fluid (or more fluid) phases in the cell. I always think it helpful in this connection to think of the least differentiated of animal cells—to consider, for instance, the amoeba. In this creature a fluid phase comes definitely into view with the appearance of the food vacuole. In this vacuole digestion goes on, and there can be no doubt, from the suggestive experimental evidence available, that a digestive enzyme, and possibly two successive enzymes (a pepsin followed by a trypsin) appear in it. It is now generally admitted that digestion in the amoeba, though intracellular, is metaplasmic. The digestion products appear first of all in simple aqueous solution. Is it not unjustifiable to assume that the next step is a total "assimilation" of the products, a direct building up of all that is produced in the vacuole into the complexes of the cell? If there be any basis for our views concerning the specificity of, say, the tissue proteins, they must apply to the amoeba no less than to the higher animal, and we must picture the building-up of its specific complexes as a selective process. The mixture of amino-acids derived from the proteins of the bacteria or other food eaten by it may be inharmonious with their balance in the amoeba. Some have to be more directly dealt with, by oxidation or otherwise. If the digestive hydrolysis occur outside the complexes, we may most justifiably assume that other preparative processes also occur outside them. We need not think of a visible vacuole as the only seat of such changes. Similar fluid phases in the cell may elude the microscope, and the phenomena would be just as significant if reactions occur in the

water imbibed by the colloids of the cell or present in the intra-micellar spaces of the bioplasm. It is always important to remember that 75 per cent. of the cell substance consists of water.

All of these considerations we may apply to the tissue cells of the higher animal. To my mind, at least, the following considerations appeal. It is noteworthy that all the known complexes of the cell—the proteins, the phosphorus complexes, the nucleic acids, etc.—are susceptible to hydrolysis by catalytic agents, which are always present, or potentially present. If the available experimental evidence be honestly appraised, it points to the conclusion that only to hydrolytic processes are the complexes unstable. Under the conditions of the body they are, while intact, resistant to other types of change, their hydrolytic products being much more susceptible. Since hydroclastic agents are present in the cell we must suppose that there is, at any moment, equilibrium between the complexes and their water-soluble hydrolytic products, though the amount of the latter present at any moment may be very small. Now, I think we are entitled to look upon assimilation and dissimilation, when very strictly defined, as being dependent upon changes in this equilibrium alone. They are processes of condensation and hydrolysis respectively. Substances which are foreign to the normal constitution of the complexes—and these comprise not only strictly extraneous substances, but material for assimilation not yet ready for direct condensation, or metabolites which are no longer simple hydrolytic products—do not enter or re-enter the complexes. They suffer change within the cell, but not as part of the complexes. When, for instance, a supply of amino-acids transferred from the gut reaches the tissue cell, they may be in excess of the contemporary limits of assimilation; or, once more, individual acids may not be present in the harmonious proportion required to form the specific proteins in the cell. Are we to suppose that all nevertheless become an integral part of the complexes before the harmony is by some mysterious means adjusted? I think rather that the normality of the cell proteins is maintained by processes which precede actual condensation or assimilation. Conversely, when the cell balance sets towards dissimilation, the amino-acids liberated by hydrolysis suffer further change outside the complexes. So when a foreign substance, say benzoic acid, enters the cell, we have no evidence, experimental or other, to suggest that such a body ever becomes an integral part of the complexes. Rather does it suffer its conjugation with glycine in the fluids of the cell. So also with cases of specific chemical manufacture in organs. When, for instance, adrenaline—a simple, definite crystalline body—appears in the cells of the gland which prepares it, are we to suppose that its molecule emerges in some way ready-made from the protein complexes of the gland, rather than that a precursor derived from a normal hydrolytic product of these proteins or from the food supply is converted into adrenaline by reactions of a comprehensible kind, occurring in aqueous solution, and involving simple molecules throughout? While referring to adrenaline, I may comment upon

the fact that the extraordinarily wide influence now attributed to that substance is a striking illustration of the importance of simple molecules in the dynamics of the body.

It should be, of course, understood, though the consideration does not affect the essential significance of the views I am advancing, that the isolation of reactions in particular phases of the cell is only relative. I have before emphasised the point that the equilibrium of the whole system must, to a greater or less degree, be affected by a change in any one phase. A happening of any kind in the fluid phases must affect the chemical equilibrium and, no less, the physico-chemical equilibrium, between them and the complexes or less fluid phases. A drug may have an "action" on a cell, even though it remain in solution, and it may have a specific action because its molecular constitution leads it to intrude into, and modify the course of, some one, rather than any other, of the numerous simple chemical reactions proceeding in the cells of different tissues.

But I must now turn from consideration of the reactions themselves to that of their direction and control. It is clear that a special feature of the living cell is the organisation of chemical events within it. So long as we are content to conceive of all happenings as occurring within a biogen or living molecule all directive power can be attributed in some vague sense to its quite special properties.

But the last fifteen years have seen grow up a doctrine of a quite different sort which, while it has difficulties of its own, has the supreme merit of possessing an experimental basis and of encouraging by its very nature further experimental work. I mean the conception that each chemical reaction within the cell is directed and controlled by a specific catalyst. I have already more than once implicitly assumed the existence of intracellular enzymes. I must now consider them more fully.

Considering the preparation made for it by the early teaching of individual biologists, prominent among whom was Moritz Traube, it is remarkable that belief in the *endo-enzyme* as a universal agent of the cell was so slow to establish itself, though in the absence of abundant experimental proof scepticism was doubtless justified. So long as the ferments demonstrated as being normally attached to the cell were only those with hydroclastic properties, such as were already familiar in the case of secreted digestive ferments, the imagination was not stirred. Only with Buchner's discovery of zymase and cell-free alcoholic fermentation did the faith begin to grow. Yet, a quarter of a century before, Hoppe-Seyler had written (when discussing the then vexed question of nomenclature, as between organised and unorganised "ferments"): "The only question to be determined is whether that hypothesis is too bold which assumes that in the organism of yeasts there is a *substance* [the italic are mine] that decomposes sugar into alcohol and  $\text{CO}_2$ . . . . I hold the hypothesis to be *necessary* because fermentations are chemical events and must have chemical causes. . . ." If in the last sentence of this quotation we substitute for the word "fermentations" the words

"the molecular reactions which occur within the cell" Hoppe-Seyler would, I think, have been equally justified.

Remembering, however, the great multiplicity of the reactions which occur in the animal body, and remembering the narrow specificity in the range of action of an individual enzyme, we may be tempted to pause on contemplating the myriad nature of the army of enzymes that seems called for. But before judging upon the matter the mind should be prepared by a full perusal of the experimental evidence. We must call to mind the phenomena of autolysis and all the details into which they have been followed; the specificity of the proteolytic ferments concerned, and especially the evidence obtained by Abderhalden and others, that tissues contain numerous enzymes, of which some act upon only one type of polypeptide, and some specifically on other polypeptides. We must remember the intracellular enzymes that split the phosphorus complexes of the cell; the lipases, the amylases, and the highly specific invert ferments, each adjusted to the hydrolysis of a particular sugar. We have also to think of a large group of enzymes acting specifically upon other substances of simple constitution, such as the arginase of Kossel and Dakin, the enzyme recently described by Dakin which acts with great potency in converting pyruvic aldehyde into lactic acid, and many others. Nothing could produce a firmer belief in the reality and importance of the specialised enzymes of the tissues than a personal repetition of the experiments of Walter Jones, Schittenhelm, Wiechowski and others, upon the agents involved in the breakdown of nucleic acids; each step in the elaborate process involves a separate catalyst. In this region of metabolism alone a small army of independent enzymes is known to play a part, each individual being of proven specificity. The final stages of the process involve oxidations which stop short at the stage of uric acid in man, but proceed to that of allantoin in most animals. It is very instructive to observe the clean, complete oxidation of uric acid to allantoin, which can be induced *in vitro* under the influence of Wiechowski's preparations of the uric acid oxidase, especially if one recalls at the same time, in proof of its physiological significance, that this oxidase, though always present in the tissues of animals which excrete allantoin, is absent from those of man, who does not.

I will not trouble you with further examples. We have arrived, indeed, at a stage when, with a huge array of examples before us, it is logical to conclude that *all metabolic tissue reactions are catalysed by enzymes*, and, knowing the general properties of these, we have every right to conclude that all reactions may be so catalysed in the synthetic as well as in the opposite sense. If we are astonished at the vast array of specific catalysts which must be present in the tissues, there are other facts which increase the complexity of things. Evidence continues to accumulate from the biological side to show that, as a matter of fact, the living cell can acquire *de novo* as the result of special stimulation new catalytic agents previously foreign to its organisation.

It is certain, from very numerous studies made upon the lower organisms, and especially upon bacteria, that the cell may acquire new chemical powers when made to depend upon an unaccustomed nutritive medium. I must be content to quote a single instance out of many. Twort has shown that certain bacteria of the coli-typhosus group can be trained to split sugars and alcohols which originally they could not split 'at all. A strain of *B. typhosus*, which after being grown upon a medium containing dulcitol had acquired the power of splitting this substance, retained it permanently, even after passage through the body of the guinea-pig, and cultivation upon a dulcitol-free medium. Similar observations have been made upon the continent by Massini and Burri; the latter showed by ingenious experiments that all the individuals of a race which acquires such a new property have the same potency for acquiring it. No one, at the present time, will deny that the appearance of a new enzyme is involved in this adjustment of the cell to a new nutritive medium.

We have not, it is true, so much evidence for similar phenomena in the case of the higher animals. The milk-sugar splitting ferment may be absent from the gut epithelium before birth, and in some animals may disappear again after the period of suckling, but here we probably have to do with some simple alternation of latency and activation. But among the "protective" ferments studied by Abderhalden we have, perhaps, cases in which specific individuals appear *de novo* as the result of injecting foreign proteins, etc., into the circulation. Consider, moreover, the case of the reactions called out by simpler substances. We have seen that an enzyme separable from the kidney tissue can catalyse the synthesis no less than the breakdown of hippuric acid. Now the cells of the mammalian kidney have always had to deal with benzoic acid or chemical precursors of benzoic acid, and the presence of a specific enzyme related to it is not surprising. But living cells are not likely ever to have been in contact with, say, bromo-benzol, until the substance was administered to animals experimentally. Yet a definite reaction at once proceeds when that substance is introduced into the body. It is linked up, as we have seen, with cysteine. Now, this reaction is not one which would proceed in the body uncatalysed; if it be catalysed by an enzyme, all that we know about the specificity of such agents would suggest that a new one must appear for the purpose. I have allowed myself to go beyond ascertained facts in dealing with this last point. But once we have granted that specific enzymes are real agents in the cell, controlling a great number of reactions, I can see no logical reason for supposing that a different class of mechanism can be concerned with any particular reaction.

If we are entitled to conceive of so large a part of the chemical dynamics of the cell as comprising simple metaplasmic reactions catalysed by independent specific enzymes, it is certain that our pure chemical studies of the happenings in tissue extracts, expressed cell juices, and the like, gain enormously in meaning and significance. We make a real step forward when

we escape from the vagueness which attaches to the "bioplasmic molecule" considered as the seat of all change. But I am not so foolish as to urge that the step is one towards obvious simplicity in our views concerning the cell. For what indeed are we to think of a chemical system in which so great an array of distinct catalysing agents is present or potentially present; a system, I would add, which when disturbed by the entry of a foreign substance regains its equilibrium through the agency of new-born catalysts adjusted to entirely new reactions? Here seems justification enough for the vitalistic view that events in the living cell are determined by final as well as by proximate causes, that its constitution has reference to the future as well as the past. But how can we conceive that any event called forth in any system by the entry of a simple molecule, an event related qualitatively to the structure of that molecule, can be of other than a chemical nature? The very complexity, therefore, which is apparent in the catalytic phenomena of the cell to my mind indicates that we must have here a case of what Henri Poincaré has called *la simplicité cachée*. Underlying the extreme complexity we may discover a simplicity which now escapes us. If so, I have of course no idea along what lines we are to reach the discovery of that simplicity, but I am sure the subject should attract the contemplative chemist, and especially him who is interested and versed in the dynamical side of his subject. If he can arrive at any hypothesis sufficiently general to direct research he will have opened a new chapter of organic chemistry—almost will he have created a new chemistry.

It must not be supposed that I am blind to the fact that the phenomena of the cell present a side to which the considerations I have put before you do not apply. Paul Ehrlich, in his recent illuminating address to the International Congress of Medicine, remarked that if, in chemistry, it be true that *Corpora non agunt nisi liquida*, then, in chemotherapy, it is no less true that *Corpora non agunt nisi fixata*. Whatever precisely may be involved in the important principle of "fixation" as applied to drug actions, it remains, I think, true that the older adage applies to the dynamic *reactions* which occur in the living cell. But there are doubtless dynamic phenomena in which the cell complexes play a prominent part. The whole of our doctrine concerning the reaction of the body to the toxins of disease is based upon the fact that when the cell is invaded by complexes other than those normal to it, its own complexes become involved. I must not attempt to deal with these phenomena, but rather proceed to my closing remarks. I would like, however, just to express the hope that the chemist will recognise their theoretical importance. He will not, indeed, be surprised at the oligodynamic aspects of the phenomena, startling as they are. When physico-chemical factors enter into a phenomenon the influence of an infinitely small amount of material may always be expected. It is a fact, for instance, as Dr. W. H. Mills reminds me, that when a substance crystallises in more than one form it may be quite impossible to obtain the less stable forms of

its crystals in any laboratory which has been "infected" with the more stable form, even though this infection has been produced by quite ordinary manipulations dealing with the latter. Here, certainly, is a case in which the influence of the infinitesimal is before us. But what I feel should arrest the interest of the chemist is the remarkable mingling of the general with the particular which phenomena like those of immunity display. In the relations which obtain between toxin and anti-toxin, for example, we find that physico-chemical factors predominate, and yet they are associated to a high degree with the character of specificity. The colloid state of matter, as such, and the properties of surface determine many of the characteristics of such reactions, yet the chemical aspect is always to the front. Combinations are observed which do not seem to be chemical compounds, but rather associations by adsorption; yet the mutual relations between the interacting complexes are in the highest degree discriminative and specific. The chemical factor in adsorption phenomena has, of course, been recognised elsewhere; but in biology it is particularly striking. Theoretical chemistry must hasten to take account of it. The modern developments in the study of valency probably constitute a step in this direction.

It is clear to everyone that the physical chemist is playing, and will continue to play, a most important part in the investigation of biological phenomena. We need, I think, have no doubt that in this country he will turn to our problems, for the kind of work he has to do seems to suit our national tastes and talents, and the biologist just now is much alive to the value of his results. But I rather feel that the organic chemist needs more wooing and gets less, though I am sure that his aid is equally necessary. In connection with most biological problems, physical and organic chemists have clearly defined tasks. To take one instance. In muscle phenomena it is becoming every day clearer that the mechanico-motor properties of the tissue, its changes of tension, its contraction and relaxation, depend upon physico-chemical phenomena associated with its colloidal complexes and its intimate structure. Changes in hydrogen-ion concentration and in the concentration of electrolytes generally, by acting upon surfaces or by upsetting osmotic equilibria, seem to be the determining causes of muscular movement. Yet the energy of the muscle is continuously supplied by the progress of organic reactions, and for a full understanding of events we need to know every detail of their course. Here then, as everywhere else, is the need for the organic chemist.

But I would urge upon any young chemist who thinks of occupying himself with biological problems, the necessity for submitting for a year or two to a second discipline. If he merely migrate to a biological institute, prepared to determine the constitution of new products from the *animal* and study their reaction *in vitro*, he will be a very useful and acceptable person, but he will not become a biochemist. We want to learn how reactions run in the organism, and there is abundant evidence to show how little a mere knowledge of the constitution of substances, and a consideration

of laboratory possibilities, can help on such knowledge. The animal body usually does the unexpected.

But if the organic chemist will get into touch with the animal, it is sure that the possession of his special knowledge will serve him well. Difficulties and peculiarities in connection with technique may lead the professor of pure chemistry to call his work amateurish, and certainly his results, unlike those of the physical chemist, will not straightway lend themselves to mathematical treatment. He may himself, too, meet from time to time the spectre of Vitalism, and be led quite unjustifiably to wonder whether all his work may not be wide of the mark. But if he will first obtain for us a further supply of valuable qualitative facts concerning the reactions in the body, we may then say to him, as Tranio said to his master:

“The mathematics and the metaphysics  
Fall to them as you find your stomach serves you.”

All of us who are engaged in applying chemistry and physics to the study of living phenomena are apt to be posed with questions as to our goal, although we have but just set out on our journey. It seems to me that we should be content to believe that we shall ultimately be able at least to describe the living animal in the sense that the morphologist has described the dead; if such descriptions do not amount to final explanations, it is not our fault. If in “life” there be some final residuum fated always to elude our methods, there is always the comforting truth to which Robert Louis Stevenson gave perhaps the finest expression, when he wrote:

“To travel hopefully is better than to arrive,  
And the true success is labour.”

N.B.—Certain passages in this address have been italicised by the Editors.

## NEWER STANDPOINTS IN THE STUDY OF NUTRITION

[*Trans. Chem. Soc.* 1916, 109, 629]

An invitation to lecture before the Chemical Society is for me a great personal honour. I feel, moreover, as a professed biochemist, much gratification from the circumstance that one of my calling has been chosen for the honour. It is, indeed, a source of no small satisfaction to those who have to apply chemistry to the animal to feel that during recent years the pure chemist has displayed a growing sympathy with that particular application of the science. A few years ago the sympathy was, frankly, less.

Properly to appreciate any branch of inquiry one must fully understand its aims, and, by experience or otherwise, one must have obtained faith in its methods. Now, in the application of chemistry to medicine and physiology, both aims and methods are more remote from the everyday thoughts and occupations of the pure chemist than in any other branch of inquiry to which his science is applied. This is, of course, only true when the living animal body is itself being dealt with; not when its foods, its constituents, or its products are under study in the laboratory; but it is only as a branch of science directly concerned with the animal that biochemistry can claim to be a separate discipline deserving of a special name. A certain sense of remoteness was, I think, the sufficient cause for the circumstance that the biochemist used to feel at some disadvantage when addressing an audience of those who profess the pure science.

Now, however, and especially this evening, I feel that I can count upon your sympathy, even though I have selected for my address a somewhat special and limited—though not, I think, unimportant—aspect of my subject. I am sure you will appreciate the aims of the inquiry with which I shall deal, and I hope you will have some faith in the methods used.

I propose to discuss, on certain special and limited lines, the fate of protein nutriment as it is dealt with in the animal body.

Any attempt to study this very fundamental aspect of nutrition from clear chemical points of view seemed, twenty years ago, to be almost hopeless. At that time, our knowledge of the chemistry of proteins was, of course, very incomplete, and the student of metabolism owes a heavy debt to the chemical labours which during recent years have done so much to throw light on the constitution of the protein molecule. This debt is largely—but by no means so exclusively as some people seem to think—due to Emil Fischer and his co-workers.

The chemical knowledge of proteins which we now possess is sufficient to increase very greatly our understanding of the processes which occur in the animal. It required, however, an advance on the physiological side—the discovery of a peculiarity in metabolism which to the biochemist represents a very fortunate aspect of affairs—before the prospect for experimental study could be so favourable as we may now claim it to be.

If molecular reconstructions, oxidations, and the other more significant chemical events which occur in nitrogenous metabolism were wholly, or in the main, initiated in the intact molecule of protein itself, the complexity of this, and the consequent complexity of, at any rate, all the earlier products of change, would still leave the chemical investigator with a task of extreme difficulty. The advance on the physiological side, to which I have just referred, has made it clear, however, that complete hydrolysis precedes other processes of change. The complex protein molecule is dissected before it is used, whether for reconstruction, for supplying energy, or for other essential purposes. The animal body makes play with the constituent amino-acids of protein, and these are substances with clear-cut chemical properties based upon known molecular constitution.

It is scarcely necessary to remind you that on hydrolysis a typical protein breaks down, by resolution of the polypeptide linkings in its molecule, into an assembly of  $\alpha$ -amino-acids of which some eighteen are known. We may not yet know all of them, but there is a good deal of evidence to show that we do know nearly all, and of those which have been separated, the constitution is for the most part fully known. There is no need for me to assemble the molecular formulae of all these units before you; they are too familiar. There is so much significance, however, in the diversity of molecular structure which obtains among them, that I venture here to remind you of that diversity. You will remember that a number of them are aliphatic substances of various types: monocarboxylic and dicarboxylic acids; monoamino- and diamino-derivatives; some containing straight chains, others branches. One is a thio-compound, another contains the guanidine grouping. A number, on the other hand, are aromatic compounds, some being monocyclic, others heterocyclic; the benzene, the indole, and iminazole (glyoxaline) ring having each one or more representatives. The more clear the evidence that in metabolism the body deals with individual amino-acids, the more significant does this diversity become. The fate of an organic substance in a given chemical milieu depends primarily upon its own molecular structure, and even amid the chemical complexities of the body properties due to such structure must still assert themselves. Substances so diverse must suffer diverse fates. The catabolism of protein follows not one line, but many lines. It is important, indeed, to realise that when we eat any individual protein, we are in effect taking into ourselves, not one substance, but well-nigh twenty substances, each possessing its own significance—sometimes greater, sometimes less—in the processes of nutrition.

I wish, therefore, to put before you very briefly, but as clearly as possible, the evidence upon which is based our present belief that before the proper metabolism of protein begins, its constituent amino-acids are liberated. This part of my subject will be familiar to many here. It may not be so to all, however, and it is essential to the development of my main exposition that the evidence referred to should be weighed, and, I hope, accepted.

I must deal first with evidence concerning the dealings of the body with a supply of new protein—with the proteins of its food. Later I will refer to the fate of the protein which has already formed part of the living tissues in so far as this is metabolised. The first thesis for which I will discuss the experimental support is this: that during digestion free amino-acids, and not complex polypeptide compounds, enter the blood from the intestine; in other words, that the digestion of proteins in the alimentary tract is a case of complete hydrolysis.

For this belief, we have first of all some indirect evidence of a very interesting sort: I mean the fact that the animal may display quite normal nutrition when depending on protein which has been totally hydrolysed before it is eaten. When a mixture of all the free amino-acids obtainable from protein has entirely replaced the normal intact protein of their food, an adult animal maintains itself, and a young animal grows. The experimental proof of this fact is abundant. It began at the hands of Otto Loewi in 1902. Various other workers have carried the proof further, and Abderhalden has accumulated evidence which is almost in excess of what is required for conviction. I will mention only one actual experiment, the last published by Abderhalden in this connection. The material fed to the animal in this experiment was so thoroughly predigested as to contain no trace of any intact polypeptide compound whatever, as proved by conclusive tests. Nothing was present but the mixture of free amino-acids which results from complete artificial digestion. On this mixture as its sole nitrogenous supply a dog lived in good health for one hundred days. During this period the animal gained 10 kilos. in body-weight, and when the experiment closed, there was nothing to suggest that it might not have been continued indefinitely. Such a result is convincing enough, and, as I have implied, the fact thus proved has become a commonplace among students of nutrition. I have myself seen scores of young animals grow steadily during experimental periods when no trace of intact protein, and no other source of flesh-forming material save free amino-acids, was passing their mouths.

There is, then, abundant proof of the capacity of the animal to synthesise its tissue proteins from free amino-acids when they are eaten, and to maintain itself quite satisfactorily on them; but this, you are entitled to say, is no direct proof that it *normally* absorbs free acids and not more complex derivatives from its bowel. I would urge, however, that it is at least very suggestive evidence even for this. The animal has, we know, wonderful powers of adaptation, but the synthesis of its tissue proteins from the medley of free amino-compounds calls for elaborate chemical adjustments,

and the fact that it is accomplished with ease under the experimental conditions described suggests that the tissues are normally adjusted to the task, and that the process is always in action.

We have, however, direct evidence that it is the unconjugated acids which enter the blood from the bowel, and a proof that these, rather than elaborate complexes, arrive at the seats of utilisation in the living tissues. I must not attempt to deal with this evidence historically or more than briefly. The work of many investigators has contributed to it. I will refer only to such observations as seem to offer the most conclusive proof, and in which I have the faith which comes from personal repetition.

Poised with the question: What precisely enters the blood during the digestion of protein? the physiologist has for various reasons failed to get a really satisfactory answer by directing his observations to the progressive changes in the intestinal contents. Success has come rather by examination of the blood and tissues before and during a meal. Since, however, such questions must be put to the living (though anaesthetised) animal, the inquirer must expect to have to work on very small quantities of blood. That is his first limitation; one that makes him welcome micro-chemical methods whenever they are of proved accuracy. Another thing that the inquirer must recognise in advance is that nitrogenous metabolism is a rapid affair, especially in its early stages, and the products of digestion, once they have entered the cycle of change, are not likely to minister to his convenience by greatly accumulating anywhere. Actual separation of the products in quantitative studies is scarcely to be hoped for. Abderhalden has, it is true, by working on very large quantities of blood, shown directly that much of the non-protein nitrogen present in the blood of animals during digestion is contained in free amino-acids, and several of these he obtained in the pure state for identification, but his evidence was qualitative only. For a quantitative method of measuring variations, we must be content with something less direct.

Now it will be known to many that an American worker, D. D. van Slyke, has so perfected the technique for liberating nitrogen from the amino groups of free amino-acids by means of nitrous acid, that intelligent quantitative applications of the process have advanced our knowledge both of the constitution of proteins and of certain aspects of their metabolism. Four years ago, in conjunction with G. M. Meyer, he began to apply the method to the problem before us, and he has attained to what is, in my opinion, real success in solving it. From the polypeptide linkings, in which amino-groups of the constituent amino-acids are engaged, nitrous acid liberates no nitrogen; only a little comes off, therefore, from polypeptides which have more than a comparatively small molecular weight. The method measures free amino-groups, and an informed use of it will distinguish between free amino-acids and all other nitrogenous constituents of tissues, so as to give a satisfactory measure of the nitrogen contained in the former alone.

The results obtained by van Slyke, on applying the method to the blood and tissues of the living animal, before and during digestion, will bring conviction to those who carefully consider them, and still more to those who care to obtain them for themselves. They show that it is mainly, if not entirely, in the form of free amino-acids, and not in complex associations of these, that our nitrogenous food begins its proper metabolism. In this form it reaches the tissues, to be dealt with according to their necessities.

As a matter of fact, our present belief that nitrogen is transported from gut to tissue, and from one tissue to another, not in protein complexes, but in the comparatively small molecules which result upon the complete hydrolysis of protein, is based, not alone on the kind of evidence already discussed, but also upon a variety of considerations all pointing in the same direction. We know, to mention but a few of them, that proteins foreign to the body, such as those contained in the food we eat, induce, if introduced while intact into the blood, a remarkable set of reactions which certainly do not follow on the normal ingestion of food. We know that the more complex polypeptides (the albumoses and peptones) which result at the first stage of digestion, prove themselves foreign to the organism, if for no other reason than that, on entering the blood, they are promptly excreted in the urine. On the other hand, we know that free amino-acids, when they enter the blood at a normal rate, are promptly and normally metabolised.

In the starving animal, or in the animal with a food supply not equal to its needs, there is, of necessity, a mobilisation of the protein which has been previously built up in the tissues by condensation of circulating amino-acids. Tissues which are less essential to the vital functions of the body yield nitrogenous material to the organs which are associated with such urgent functions. In this case, also, there is scarcely a doubt that complete hydrolysis precedes all transport from one locality to another. For an understanding of this fact we have long been prepared by our knowledge of the proteoclastic enzymes (the autolytic ferments) found in living tissues, and of the factors which condition their activity. A study of the blood during abstinence is confirmative. We do not, for instance, find the specific proteins of muscle appearing in the blood when in starvation the muscles waste. We find no complex polypeptides of any kind, but we do find a supply of amino-acids always circulating; and since these are continually being metabolised, the supply can only be maintained by disintegration of the tissue proteins.

If the liberation of free amino-acids in digestion and in tissue autolysis were connected only with transport; if the reconstruction of chemically vague complexes in this or that organ always preceded the occurrence of deep-seated and significant processes in metabolism, there would not be so much immediate promise for the application of genuine chemical knowledge to the study of metabolism as certainly exists. We may assert, however, that in metabolism it is comparatively small molecules of known or

ascertainable structure that undergo the most significant changes. I will return to the support of this claim later. You will, I think, appreciate both its reality and its importance rather better when certain other facts are before you, the facts which it is the immediate purpose of this lecture to disclose.

Although all students of nutrition have been impressed by the inherent interest and importance of the circumstance that the animal can maintain itself satisfactorily on a mixture of free amino-acids, I am not sure that it is fully recognised how excellent an opportunity for discriminating study of an important side of metabolism it has put into our hands. Since the animal flourishes on a mixture which can be fractionated, we are at once able, for example, to determine what is the effect on growth and health of withdrawing this or that individual amino-acid from the supply. We can see whether, in any particular case, their withdrawal deprives the animal of something essential, or whether, perhaps, the removal from the food of any particular amino-acid under study may have little effect because the animal can synthesise it for itself or dispense with it altogether.

We can thus measure the relative nutritive importance of different molecular groups in the protein molecule. We may determine, again, how far one amino-acid is replaceable by another more or less closely related to it, and so test the limits of the capacity of metabolism to remodel a molecule of the kind. But we may hope to go further, and discover, by a more intimate study of the precise effects of its absence, whether a given amino-acid, already proved indispensable, possesses some special function—something other than what it contributes as a unit to the building-up of tissue protein—some function depending on its special molecular structure and not to be subserved by any of its congeners. We may ultimately be able to say what are the qualitative as well as the quantitative limitations to an efficient amino-acid supply, and what is the most harmonious balance of amino-acids for the nutrition of one animal species or another. Some of these points may be only of academic interest; others are certainly practical, and all are interesting. Having for control material an efficient nutritive mixture of known and comparatively simple substances derived from protein, we have the opportunity, on the above and other lines, of so altering the balance and harmony of the mixture as to throw much light on the intricacies of nutrition by protein; an opportunity which would certainly be absent if the animal body used as the raw material for its alchemy only the complex molecules of the intact proteins themselves.

Such a line of experimental study has still; of course, difficulties of its own. When protein has been hydrolysed, there are technical difficulties in connection with the quantitative removal of some of the amino-acids from the mixture obtained. These will certainly be overcome, however, if the stimulus of interest in the subject prove sufficient. There will always be difficulties arising from the extreme adaptability to circumstances displayed by the living animal, which tends to obscure certain issues. But

this adaptability has very definite limits, or there would be no science of nutrition. It is, after all, part of the essence of the problem to study and explain this adaptability and, when necessary, in experimental studies, to learn how to elude its effects.

Some preliminary experiments on the lines just sketched I am now to describe. They come mainly, but by no means exclusively, from my own laboratory. Most of them were carried out on animals no more noble than rats, and I am not sure whether I ought not in parenthesis to enter upon some defence of studies made on rats. The fundamentals of nutrition are the same in all mammals. Specific differences are displayed only in details which can always be controlled and allowed for. I would be as sure of finding facts observed from the physiological behaviour of rats confirmed in the human body as any won from the study of larger animals; sooner, indeed, than when the animal is one of the large ruminants of the farm. As a matter of fact, the rat offers exceptional advantages for the study of certain aspects of nutrition. Apart from the fact that it is omnivorous, its comparatively small demands have a great advantage when the experimental dietary requires much labour for its preparation. On the other hand, in special connections, rats may with convenience be fed in large numbers, and statistical material thus obtained for the elimination of individual variations. Again, the small bodies of such animals are associated with so rapid a metabolism that indications of modified nutrition are quickly displayed. Last, and not least, we now possess, chiefly as the result of American work, extraordinarily accurate and very abundant data relating to the normal curve of growth and to other physiological constants of the rat. Needless to say, in nutritional experiments, as in all other experimental work, some preliminary knowledge of the peculiarities of one's material is a prime requisite for success. The rat, properly used, offers, as a matter of fact, highly standardisable material.

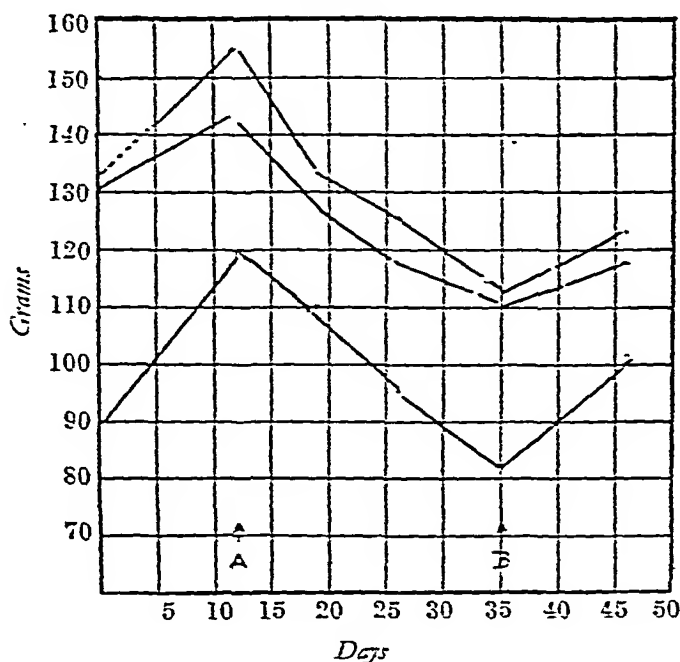
I will first put before you the body-weight curves of young rats to demonstrate the effect on the growth function of withdrawing this or that amino-acid from the food eaten. The diets which the animals were receiving comprised in all cases amino-acid mixtures combined with suitable amounts of filtered butter-fat or lard, potato starch, cane-sugar, and the requisite inorganic salts. In all the experiments which I shall deal with the diet was identical, except for the variation in the content of amino-acids. During the use of such synthetic diets, it is always essential to supply the at present mysterious vitamine or food hormone factor, which I myself, among others, have shown to be quite essential to growth. In the present experiments, this was supplied in the form of minute amounts of a nitrogen-free alcoholic extract of fresh milk. When the amino-acid mixture, which was always the sole source of nitrogen, comprised the whole assembly contained in a typical protein, growth was always maintained, and the rate of growth on this diet usually served as the normal from which the effect of deficiencies was measured. The technique of preparing the amino-acid

mixture was of the simplest. Casein was completely hydrolysed by forty hours' boiling with 25 per cent. sulphuric acid. The acid was removed and the solution of amino-acids evaporated to dryness. Acid hydrolysis destroys one amino-acid, and with completeness, one only. Tryptophan, which contains the indole nucleus, is wholly destroyed. Cystine is apparently partly destroyed. If to the products of acid hydrolysis the requisite amount (about 2 per cent. of the whole mixture) of tryptophan together with a little cystine be added, we obtain a product which satisfactorily supports growth.

That a supply of tryptophan is necessary to the animal was first indicated by some work done by Miss Willcock (Mrs. Stanley Gardiner) and I, so far back as 1907. Abderhalden has since confirmed the fact on dogs. The circumstances just mentioned make it, I find, especially easy to prepare comparative dietaries showing the effect of the presence or absence of tryptophan. One has simply to add it or to refrain from adding it to the mixture obtained on acid hydrolysis, before making up the experimental dietary.

The curves of Fig. 1 bring out in a striking way the nutritive importance of tryptophan.

FIG. 1.



Showing effect on growth of the presence or absence of tryptophan.

The first ascending part of the curve shows the growth of animals when on the whole amino-acid mixture. At the point A they were put on food from which tryptophan was absent. Loss of weight is seen to follow

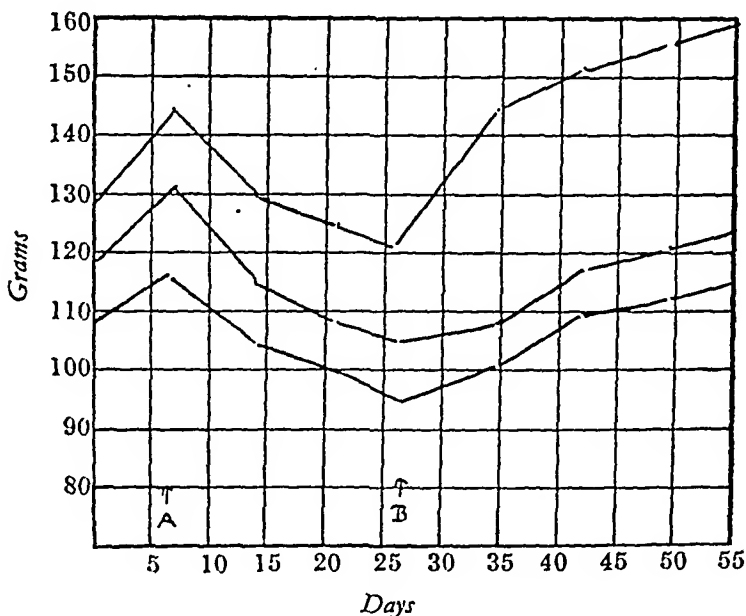
immediately, and if the animal is kept on such a diet, decline of weight continues and death follows, even though the consumption of food is, in the quantitative sense, more than sufficient for proper maintenance. On the other hand, growth will begin again if the tryptophan be restored before it is too late. This restorative effect is shown in the curve from the point *B* onwards.

It should be understood that the deficiency producing the loss of weight exhibited in all the curves to be shown is always a qualitative and not a quantitative one. In none of the experiments put before you was the animal at any time eating less than enough to give it an efficient supply of energy.

The need of a supply of tryptophan in the diet is urgent, as a great number of experiments have convinced me. The animal seems unable to synthesise the indole ring out of other materials. The necessary amount of tryptophan, however, is only about 30 milligrams a day in the case of an individual rat.

In the next curves (Fig. 2) is shown the effect on growth of quite another deficiency.

FIG. 2.



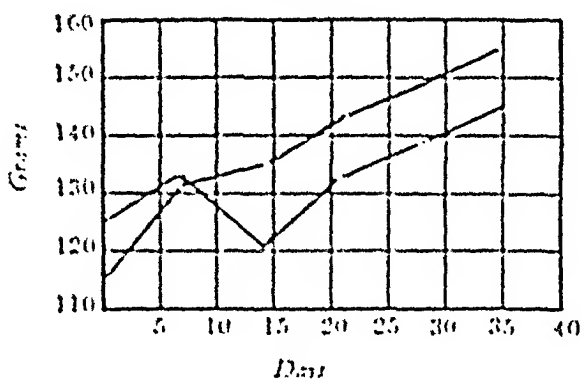
At *A* arginine and histidine were removed from the diet; at *B* they were restored.

From the amino-acid mixture fed to the animals in the experiment which yielded this curve of body-weight, arginine and histidine were both removed by the method originally described by Kossel & Kutscher for the estimation of these two amino-acids. Why the deficiency was here made a double one will become clear a little later. As soon as these two complex amino-acids were removed from the diet, growth, as the curves show, gave place to loss of weight; on their restoration, growth began again.

It is evident, then, that the simultaneous removal from the normal amino-acid mixture of the units which contain the guanidine and iminazole groupings, greatly affects the nutrition of the animal. It needs these molecular groups, and is either unable to synthesise them from other material, or its synthetic powers cannot keep pace with its own normal demands.

This may not be so in the case of other amino-acids, and would seem to be certainly otherwise in the case of some which possess a comparatively simple molecular structure. The following curves show that growth can occur in the absence of both glutamic and aspartic acids. F. W. Foreman has recently described a method for isolating these two acids from the mixture obtained on protein hydrolysis, and he himself removed them from the material which was used in the diet of animals exhibiting the growth shown in the curves of Fig. 3.

FIG. 3.



Showing the growth in the absence of glutamic and aspartic acids.  
On the seventh day these acids were together removed from the food.

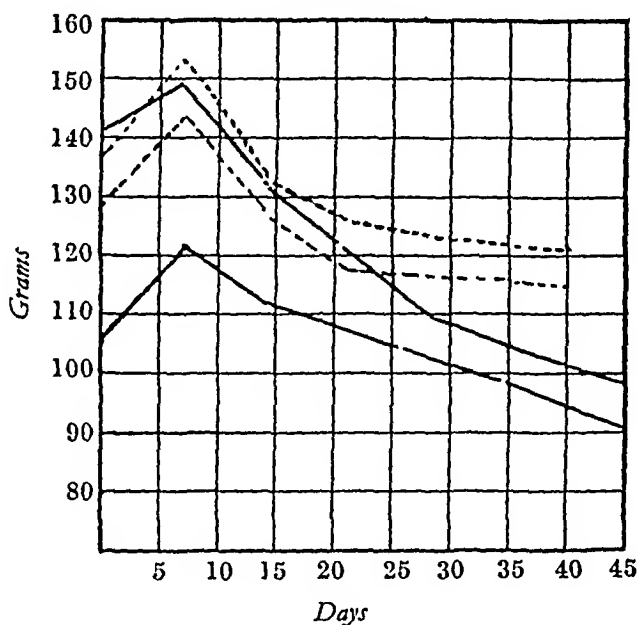
Now, in casein, glutamic and aspartic acids together constitute no less than some 28 per cent. of the weight of the molecule, and it is striking to see that by no means unsatisfactory growth was obtained in the absence of such prominent constituents of protein. One animal added 35 per cent. to its body-weight during a period of four weeks. In such a case we are not, I think, to suppose that the process of new tissue formation dispenses with constituents which, like glutamic and aspartic acid, are normal to the proteins of the tissues. We have much evidence against this supposition. We are rather to believe that the simple straight chain aliphatic acids can, in the presence of ammonium salts, be synthesised from fat or carbohydrate derivatives by reactions possessed of sufficient velocity to maintain a supply for the purposes of growth.

The contrast between the effect of removing tryptophan, which constitutes only about 1½ per cent. of the casein molecule, and that of removing

glutamic and aspartic acids, which constitute nearly 30 per cent., is sufficiently striking.

It is interesting to observe how curves of body-weight can demonstrate that, in the demand for amino-acids which seem essentially indispensable, there are, so to speak, degrees of urgency. The absence of one acid may, for example, cause a more rapid loss of weight than that of another. In Fig. 4 are curves which show once more the loss of weight which follows the absence of tryptophan, now compared with others showing the loss due to the absence of arginine and histidine.

FIG. 4.



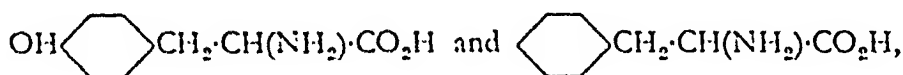
Continuous line, absence of tryptophan; dotted line, absence of arginine and histidine.

In all cases (I have selected only a few out of many observations), the lack of the indole derivative produces the more marked effect; loss of flesh is more rapid and continuous than when the guanidine and iminazole derivatives are concerned. This difference, like the other results that I am putting before you, can be obtained with great constancy. At the moment, we have not the data upon which to base an explanation of such a difference in this or in any similar case. It may depend only on the relative amounts of this or that acid, which tissue breakdown is capable of supplying in a given time. It is possible and more likely that such an amino-acid as tryptophan may be required for something other than structural purposes: that the indole nucleus, for instance, ministers to some dynamic function in the body of more urgent importance than any purpose subserved by, say, arginine and histidine. If so, we can understand how tissue breakdown would be the more rapid in order to maintain a supply for the more urgent

demand. On the other hand, it is possible that the difference depends on factors which are more purely chemical. The animal body has much more extensive synthetic powers than used to be ascribed to it; but it has more limitations than the plant in this respect. The formation of the indole ring would seem, as I have said, to be beyond its powers. It cannot, at any rate, call on reactions for its production which have sufficient velocity. On the other hand, we have some grounds—chemical grounds, at least—for believing that the body may synthesise the iminazole ring, and, if so, because of a physiological relation to which I shall shortly allude, it may also improvise the guanidine complex. These two complexes, like others contained in protein, it normally receives, directly or indirectly, from the plant by taking them in its food. With a failure in this supply, however, may come such an alteration in tissue equilibrium that reactions, always possible and imminent, or, more probably, always occurring to some small extent, proceed at an increased rate. Speaking teleologically, we might say that the living cells call up their chemical reserves. The normal position is not recaptured, but disaster is diverted or delayed. These are pure speculations, but one's indulgence in them is justified by other experimental facts, besides the one before us. I only mention them here to illustrate the kind of problem that arises in the course of a detailed study of nutrition; problems which are, in essence, chemical.\*

Although, as I have emphasised, great diversity obtains among the amino-acids which arise during the digestion of protein, some of them are more or less closely related in respect of chemical structure to certain others; and it would seem that such related acids may have some degree of equivalence in the processes of nutrition. With either, but not with both, of a given pair, the animal can, it would seem, more or less conveniently dispense.

As is well known, all typical proteins yield both tyrosine and phenylalanine:



which differ merely by the presence or absence of the phenol hydroxyl group.

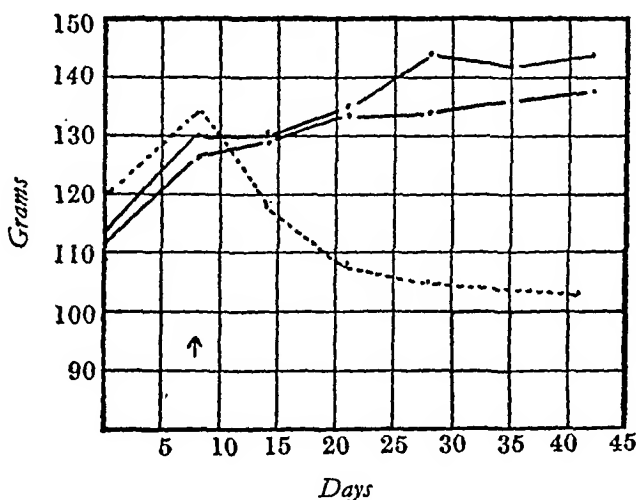
Dr. G. Totani set himself in my laboratory to test whether or not tyrosine would prove to be an indispensable constituent of the nutritive amino-acid mixture. He was met by the difficulty that in this case there is no method available for rigorously removing all that substance from the assembly of acids. He fed animals, however, on material which, as careful study showed, contained no more than a very small remainder of that present in the original protein. On this he got satisfactory growth.

I think we may safely ascribe growth in the absence of tyrosine in part,

\* It is possible that Kossel & Kutscher's method does not remove the last traces of arginine and histidine. I have been at some trouble to detect these amino-acids in the mixture as fed, but have failed to do so.

at least, to the circumstance that the phenylalanine, which was still present in the food, is more or less equivalent to tyrosine, and can cover the essential demands of the animal. That tyrosine can be formed from phenylalanine in the body is known from the experiments of Embden, who showed that when the latter is perfused through a surviving liver, the former is produced. Dr. Totani's further results, however, furnish at least a suggestion that the animal can call up some power of synthesising the benzene ring. He fed other animals on the hydrolytic cleavage products of gelatin, which contains neither tyrosine nor tryptophan. On this, of course, there was complete failure of nutrition. Nothing, I think, could make you realise more fully why gelatin does not maintain the nutrition of man or animal than experiments like those I am putting before you. The entire absence of tryptophan from its molecule stamps it at once as a deficient foodstuff.

FIG. 5.



Dotted curve shows loss of body-weight when (on the eighth day) both arginine and histidine were removed from the diet. Upper curve shows the effect when, of the two, only histidine was present; middle curve with arginine alone.

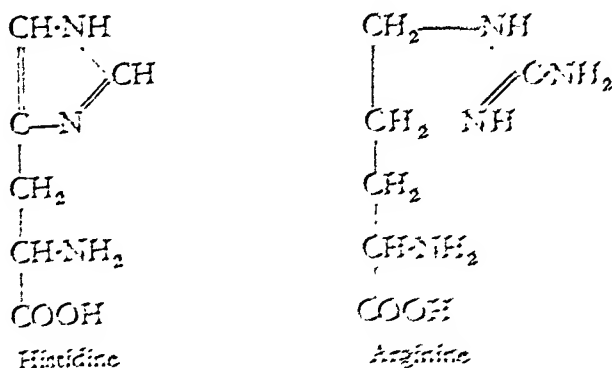
In Dr. Totani's experiments, the addition of tyrosine and tryptophan greatly improved the nutritive power of the gelatin products, as was to be expected; but what surprised us was the circumstance that many animals displayed fairly satisfactory growth after the addition of tryptophan alone; they grew, that is to say, in the absence of tyrosine and with an extraordinarily small supply of the equivalent phenylalanine. Now it must be admitted that gelatin may contain some unknown aromatic compound; we are, unfortunately, not quite sure on the point. If so, however, it is certainly present in very small amount, and there seems, as I have said, to be a suggestion in Dr. Totani's results that the animal, while unable to produce the indole ring, has some power of synthesising the benzene ring

in the course of making phenylalanine and tyrosine, which are certainly necessary for the formation of new tissue protein during growth.

I must now refer to another case of apparent equivalence between two amino-acids in metabolism; one which is of chemical as well as of physiological interest. It is a case which bears closely on other points raised in this lecture. We have seen how marked is the effect of removing arginine and histidine together. I will now show you curves of body-weight obtained when only one of the two is absent from the food. The lower curve of Fig. 5 shows once more the rapid fall when both are lacking. The middle curve illustrates the change in body-weight when of the two histidine alone is absent; the upper curve shows the result obtained when histidine is given in the absence of arginine. Instead of a rapid loss of tissue, there is, when either is present alone, something more than maintenance; there is quite appreciable growth. As a matter of fact, in the particular experiment which yielded the curves shown, the amount of the amino-acid in question was in each case much smaller than that required to restore the balance of a normal amino-acid mixture. Better growth is obtained when either substance is given in amount equal to the normal sum of both as present in casein.

Either of these two protein constituents can, apparently, subserve the functions normally subserved by both together.

This is not surprising when the structural formulae of arginine and histidine are compared in proper relation; the former is a guanidine-amino-propionic acid, the latter an iminazole-amino-propionic acid; but on putting the formulae side by side, we can see how closely they are related:



The essential molecular changes involved in passing from one to the other—the opening or closing of a ring and the addition or removal of an amino-group—we may, from our general knowledge of the chemical process of the body, look on as essentially “physiological” processes. The ready suggestion which such preliminary nutritional experiments convey, that this or that reaction occurs in the body, should be followed by chemical studies. A final appeal to the animal can then be made. We have, for example, seen how precisely on chemical lines histidine can be converted into arginine,

and how the reverse change can be brought about. We could then test the ability of the animal, or of its various organs, to guide the reactions through the necessary intermediate stages. To say the truth, the nutritional fact is, in my belief, a sufficient proof that the reactions do occur, but a chemical study is required to help us to discover on what lines they may occur, as a guide for further experiments on the animal. From the animal to the laboratory and from the laboratory back to the animal is the logical order in such researches. The pure chemical studies should gain in interest, and certainly do not lose in dignity, from the fact that they deal with processes with which living tissues are concerned.

I return, before closing, to two further aspects of my subject, which can again be illustrated by experiments with free amino-acids. We have seen that different fractions of the protein molecule have different nutritional values. The question arises: Can the animal be maintained when supplied with only a limited number of amino-acids? Can it, for example, survive if, instead of protein or all the amino-acids contained in protein, it receives only the more complex types of the latter, those which experiment shows to be outside the range of its synthetic powers. The question is doubtless somewhat academic, but it is of theoretical importance, and has one quite interesting bearing. If nutrition were possible on a quite limited number of amino-acids, we should be so much the nearer to the possibility of artificially synthesising our food supply; distant, in any practical sense, as that possibility may be. My own experiments have as yet given no conclusive answer to the question; but I have found that when an animal is fed on food containing only tryptophan, histidine, tyrosine, lysine, and cystine, that is, five out of the eighteen amino-acids of protein, there is a remarkably slow loss of weight, and long maintenance of apparent health. This result is in sharp contrast to what is seen when the five amino-acids mentioned are replaced by another five of simpler aliphatic type. When leucine, valine, alanine, and glycine, together with glutamic acid, form the sole nitrogen supply, loss of weight is rapid, and the animal soon succumbs. I feel it quite possible that further experiment will show that life can be maintained on a mixture from which many of the amino-acids of protein are lacking.

I now come to the last consideration with which I shall trouble you. In picturing the animal as it maintains itself on a supply of diverse amino-acids, we are entitled to ask, what are the various uses to which that supply is put? If the animal be a growing one, it must, of course, use a part for the construction of new tissue proteins; if an adult, for the repair of tissues. This involves a selective condensation of the various units into polypeptide complexes possessing a definite and specific stamp of structure. We have been already considering, in connection with the weight curves which I have put before you, to what degree this or that deficiency in the supply can interfere with the progress of such synthesis. A second fate suffered by the amino-acids is immediate oxidation. It is very unlikely that the supply of them from the food will ever display the exact quantitative

balance among the diverse units which will correspond with what is found in the specific tissue proteins of a given animal. During the selective regrouping, some individual acids will be in excess, and will be directly broken down on oxidative lines. In any case, protein as a whole is usually eaten in excess of the immediate needs, so that such direct oxidation is always going on. Our newer point of view as to the metabolic fate of protein lends greater interest to the study of these oxidations, because we are now aware that we have to follow the separate fate of individual amino-acids, which in each case is circumstanced by the properties of known molecular structure. My desire to impress you with the immediate applicability of pure chemical considerations to the study of animal metabolism might, indeed, have been better served had I spent my time in reminding you of the knowledge which has been already won in connection with these oxidations. There is much yet to learn about them, but patience and a good deal of real ingenuity has given us a mass of trustworthy information in what is, for the experimentalist, a very difficult domain. This ground has, however, been made familiar by the publication of certain excellent summaries, and I have preferred to deal with somewhat different aspects of the subject.

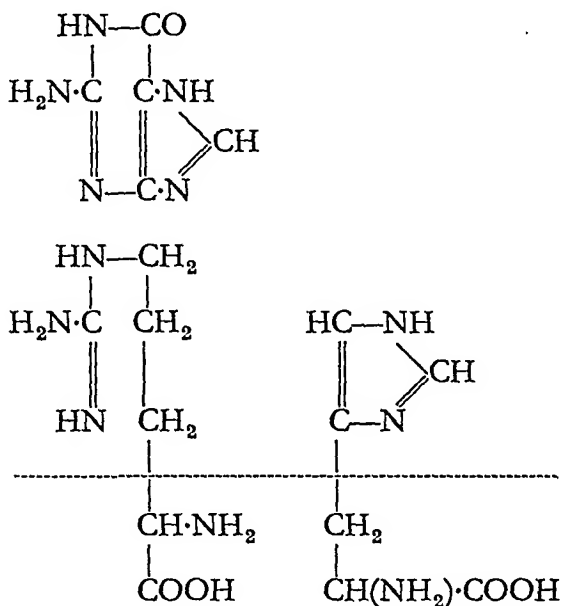
There is yet a third kind of use to which the body puts parts of its amino-acid supply. Some of the units contribute directly or indirectly to the formation or upkeep of nitrogenous tissue complexes in which we do not find the structure of polypeptides or amino-acids. Other units probably undergo transformation into the specific constituents of secretions which possess a dynamic function. We have as yet scarcely any experimental information as to what particular (if any particular) amino-acid or acids may be the raw material satisfying this or that demand of the kind mentioned. We are consequently ignorant as to the nature of the reactions involved. We know that nitrogenous constituents of the living tissues, which are not themselves of a protein nature, can be made and maintained when only protein is eaten. Amino-acids, therefore, must be the ultimate raw material for such formations; but we do not know at what sort of level synthesis begins. It may be in this or that case that the body has chemical magic enough to be somewhat indifferent to the precise nature of the raw material, but some of the facts which have been already before you indicate that such indifference is not its habit.

At any rate, when some structural relationship is evident between a food-constituent and a tissue-constituent it is justifiable to suspect that the former, and not some wholly unrelated substance, provides the raw material for the manufacture of the latter. Such considerations at least provide a guide for experiment. In this connection I want to call your attention to one other set of feeding experiments which bear on what I have just been saying.

Forming an integral part of the living cell nucleus are the polynucleotides or nucleic acids. These contain as essential units in their structure the

purine bases adenine and guanine, which the growing animal can synthesise—as abundant experiment has shown—when eating no other nitrogenous material than protein.

Now the fact that the histidine molecule contains the iminazole ring has suggested to others that it might play some special part in the formation of purines in the body, but that matter has not, in my opinion, been tested under conditions which could yield proper evidence. It may be remarked that arginine, like histidine, has one aspect of structure which is not found in any other amino-acid from protein, the presence, namely, of a carbon atom interposed between two nitrogen atoms. This is, of course, characteristic of the purine ring. It is perhaps worth while to compare the structure of arginine and histidine with that of one of the purine bases.



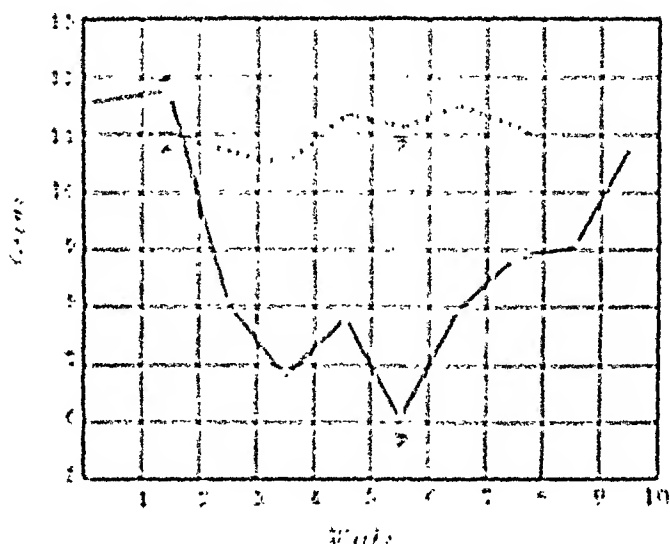
It would seem on inspection as though both these amino-acids might in some way share in the formation of the purine ring, but if, as I have claimed, the body can convert either one of them into the other, the point has small significance. It is in any case useless to speculate as to precise molecular relations in advance of chemical study; but the probabilities which exist fully justified a preliminary nutritional study, and I think I have obtained evidence on the matter which is highly suggestive, if not conclusive.

Purine metabolism in all animals, except man himself, ends with the excretion, not of uric acid as in man, but of allantoin, and if we are to determine whether any factor has influenced purine metabolism, we must estimate the allantoin of the urine. In the experiments to be referred to, the animals were first fed with a complete amino-acid mixture; then for a period arginine and histidine were withdrawn, while in the third period they were restored to the food. The urine was accumulated, and the

allantoin excretion in the excretion of successive weeks. The lower curve in Fig. 6 shows the effect of the presence or absence respectively of arginine and histidine on the allantoin excretion. There is a very marked fall as a result of their withdrawal, whilst their restoration is followed by a rise.

Such a result seems clearly to indicate a special relation between these particular amino acids and the end-product of purine metabolism. It therefore suggests that they play a special part in the formation of the purine ring, but this conclusion must not be accepted without proper experimental controls. I must not stop to discuss the point fully or critically, but I can give you the evidence which removes the only important criticism. It need be only stated that the fall in allantoin is due solely to the general depression of metabolic activity due to the removal of a factor essential to nutrition.

FIG. 6.



The continuous line shows the effect of presence or absence of arginine and histidine on the excretion of allantoin. These two amino acids were removed at A and restored at B. Dotted line shows effect of removing and restoring tryptophan.

We know already that there is a notable fall in weight when the animal receives no arginine or histidine. I have tried, however, the effect of other deficiencies which produce an equal or greater disturbance of nutrition on the excretion of allantoin. The upper curve in the figure shows the effect of removing tryptophan; this, as we know, involves complete failure of nutrition and a sharp fall in weight; but, as you will see, there is no similar effect on the excretion of allantoin. I have tried the results of other deficiencies, also with negative results. There can be little doubt that arginine and histidine play a special part in purine metabolism, but here again the full proof requires the assistance of the organic chemist. We

want next to trace the intermediate stages of the reactions which intervene in the conversion of the amino-acid into a new type of structure. I would like, indeed, to feel that the few facts and the more numerous suggestions which have been before you this evening—facts and suggestions won in a very narrow field of research—may suffice to leave behind the impression in your mind that, because intermediary metabolism deals so largely with materials of which the actual molecular structure is known or ascertainable, the trained thought of the organic chemist has abundant opportunities in connection with physiological research. We have dealt with protein metabolism alone, but it is equally true of metabolism in all its aspects. You may urge, of course, that the formation of the tissue complexes with their extreme chemical elusiveness is, after all, the most significant step in metabolism; so in a sense it is. The physical and physico-chemical properties of these complexes confer on the living cell its prominent mechano-motor properties and many of its reactivities, as well as providing the milieu in which chemical reactions occur. In my own belief, however, these complexes, once formed, are, in the chemical sense, essentially stable in all but one particular. Just as they are formed and maintained by the condensation (in the technical sense of the word) of the simpler molecules which are ever circulating in the blood, so are they liable to hydrolytic disintegration, yielding, like the food, simple materials for fresh synthesis or materials to undergo oxidation and provide energy to the body. Condensation and hydrolysis respectively lock and unlock the door which prevents or permits the occurrence of those oxidations and that regrouping of units which form the essential chapter in the chemical dynamics of metabolism. On the other side of that door there is relative chemical stability. About the details of condensation and hydrolysis in the tissues we have an indefinite amount to learn, but in connection with large aspects of metabolism they may be taken for granted. If we follow our molecules of known structure up to the door, and follow them yet again as they emerge from it, we shall learn a large part of what we want to know about the chemical dynamics of the animal body.



concern of him alone, nor his exclusive concern, have been the main preoccupations of the biologist. Hence arose what we may term biological language. It is a tongue apt for description of the visible, apt in its own way even for quantitative statement; but its currency is necessarily limited. It is a different tongue from that which has been spoken in the domain of the, so-called, exact sciences. On the other hand, the pure chemist, for a long time at least (it is otherwise now, as I shall point out), worked without concern for form and visible structure. He long studied phenomena only after they had been first reduced by his own art and methods to their simplest form, and he dealt with systems made so far as possible homogeneous. He was mainly concerned with such concepts as volume, pressure, concentration, and the like, and with a set of all important numerical relations displayed among the data he collected. So far as he visualised, it was with his mind's eye; for the objective apparatus he used was of secondary importance and accidental. His thoughts were based on the properties of the invisible and indivisible atom.

It is making, I know, a great claim, but I believe that it will be the ultimate privilege of advancing biochemistry to tempt all biologists—including the physician—always to picture mentally—as a habit of mind—the molecular events which underlie the changes of form and visible appearances which interest them, and, on the other hand, to demonstrate to chemists that the molecular events they have studied so fully in systems more or less homogeneous, gain enormously in interest, in spite of the complications involved, when they are organised and co-ordinated in systems involving changing form and elaborate structure.

If it be admitted that the ultimate aim, or at least the constant endeavour, of biochemistry as an academic pursuit should be to describe the activities of living organisms in terms of physical and organic chemistry (and I for one believe that the description will in such terms be as complete as any that can be given in their own more specialised terms by the morphologist or by the physiologist when he deals with functions in terms of organs); this description will possess the merits due to the use of a more universal scientific language. If this be the accepted end of biochemistry, as distinct from merely utilitarian ends, it is sure that as a branch of scientific inquiry it must show somewhat exceptional characteristics and make rather special demands. Its votaries are to a peculiar degree under the compulsion of obtaining most of their technical skill from one domain and most of their mental outlook from another—a circumstance concerning which I shall later say more.

Technically, of course, the subject presents difficulties depending upon the complexity and instability of the materials it must deal with; difficulties which were once held to make its efforts nugatory; difficulties which are certainly felt in the sphere of method. It is part of the purpose of these lectures, however, to indicate that in spite of these difficulties progress in the accumulation of significant facts is yearly accelerating.

There are many however who, before devoting their lives to a difficult scientific discipline, will desire to know that its progress is towards some ultimate goal which is as real as the goal of any other branch of scientific endeavour. How will they be affected by such a statement as the following: "The new physiology is biological physiology—not biophysics or biochemistry. The attempt to analyse living organisms into physical and chemical mechanism is probably the most colossal failure in the whole history of modern science"? Dr. J. S. Haldane has expressed in these words, and many times in similar words, and again, very recently, what is avowedly a profound personal conviction. It is a duty almost on the part of any biochemist to try and discover exactly what the statement means.

If it means just what it seems to mean and is justifiable, then the biochemist must retire from the field or be content with at most a pragmatic justification for his labours. I hope to convince you that a better fate is in store for him. The emphatic pronouncement just quoted is based upon no belief in vital force, but rather upon the view that the living organism as a whole is so much more than a sum of its parts that we get no further in real knowledge when we attempt to study it piecemeal. In biological thought, according to this view, the *whole* should be something axiomatic, as ultimate a conception as *etc* (or *et cetera*) the "atom" or "energy" in physics. This attitude towards the study of living things has been called *organicism*. Save in explicitness of statement it is not new, indeed, its chief exponent has traced it back to Hippocrates. Part of what it assumes tends to be in the thought of everybody concerning living organisms. No one doubts the dominant importance of those properties which are peculiar to the *whole*. The only question to decide is whether there must be so elusive and insusceptible to analysis as to justify the crushing pronouncement which I have just quoted.

If there be any tangible truth in the doctrine that all attempts to study the organism by isolating its parts for experiment are inherently futile, it is at any rate the only doctrine which to-day need give pause to the ambitions of biochemistry. The idea of a force, or entelechy, which in a living organism controls and modifies chemical events as to make unreal all attempts at chemical description, seems, after a forceful return some years ago, to be again retreating from current thought.

Relieved of this bogie the biochemist became assured (unless oppressed by the view which has been called *organicism*) that the individual processes which he studies, even when they are to some degree or other isolated from the whole organism, are still significant biological events because still controlled by factors which are biological. But if he be (as he should be) something more than a mere technician he would be indeed discouraged if he had to believe that "colossal failure" must attend any future attempt—at least in thought—to reconstruct from his ultimate data some real aspect of the organism as a whole.

No student of biochemistry can be so foolish as to forget the impressive

circumstance that the materials he works with form part, or have formed part, of a sentient organism endowed with attributes which, having neither spatial extension nor numerical values, are beyond his scope. It is not, however, because of these associations that the exponent of organicism criticises his efforts.

All that the biochemist has to be assured of, when in his laboratory, is that there is no such fundamental difference in *category* between the material events he studies and the events of inorganic nature, as to call for different methods of study or for a different descriptive language and different intellectual tools. If indeed the only kind of description open to him is to be worth anything at all it must be frankly mechanistic. He recognises, however, that the organism is in no strict sense a machine. A machine is a mechanism only of importance because adjusted to some exterior purpose, being without meaning unless it fulfils such a purpose. An organism, on the other hand, is adjusted as *a mechanism in order that it may be itself*. But this is not true of the biological organism alone. It is true of the atom of modern physics and of the complex molecule of modern organic chemistry; a circumstance which, as I hope to show, is worthy of emphasis. In so far as "mechanism" denotes an aspect of phenomena which must be visualised, mentally or otherwise, and defies immediate abstraction, physics and chemistry seemed for a time at least to be able to ignore it far more completely than any biologist could do, however much inclined to vitalist views. It is, however, a circumstance of no small interest to every biologist that the physicist and chemist are at the moment definitely concerned with mechanisms which they must visualise and describe.

It will be useful here to recall a chapter of scientific history which is comparatively recent.

Within recent years physics and chemistry seemed to be developing a viewpoint which, had it been established, would have made very difficult the immediate application of the content of those sciences to biology. In the later years of the last century there was an intellectual movement, led in particular by Wilhelm Ostwald and Ernst Mach, but supported by many others, in favour of ignoring altogether the mechanisms which underlie, or had been assumed to underlie, observed phenomena. For some years after the kinetic theory had been perfected by Clausius and Clerk-Maxwell physicists were mechanists in thought; while chemists, basing their conceptions upon the indivisible atom of Dalton and the fruitful hypothesis of Avogadro, were equally so. During the eighteen-nineties, however, there was a stirring of the scientific conscience in certain leaders of thought, and among them those just mentioned. They awoke to the fact that there was then no rigid proof of the existence of atoms and molecules, and they asked why such hypothetical entities should intrude. "Let us rather renounce," they said, "the desire to visualise events; for after all," as Ostwald once wrote, "the history of science teaches that all hypotheses involving visualisation finish by giving false pictures." The better method,

it was felt, was to obtain such quantitative data as a given phenomenon will yield, and wherever possible to enshrine these data in differential equations without reference to any mechanistic hypothesis whatever.

This, for many, must in any case have remained a counsel of perfection. There are minds, held by some to be of an inferior type, which must visualise in order to think. Duhem, one of those who supported the intellectual movement in question, remarks: "Strong minds, those who have no need of concrete images in order to comprehend an abstract idea, should not, after all, refuse to weaker spirits who find it difficult to grasp that which has neither form nor colour the right of depicting in imagination the objects presented by theoretical physics. The best mode of furthering the development of science is to allow every intellectual form to develop according to its own laws, and produce its own type; that is to say, letting the stronger spirits feed on abstract ideas and general principles, while the others seek their spiritual nourishment in visible and tangible things." Helmholtz, we may assume, confessed to a personal preference for the presentation of data in the form of differential equations without the effort of visualising, but admitted that in the thought of Kelvin and Clerk-Maxwell the effort in question was fertile enough. It would seem that (type of mind apart) the real criteria of value which must be applied to these opposed methods of thought and presentation is their relative applicability to the particular kind of phenomena under study. Ostwald, however, looked forward to a science free from hypotheses, and urged, during the period we are thinking of, that the phenomena of physics and chemistry should be presented from a standpoint which ignores mechanisms. He mistrusted at any rate the actual visualisation of atoms and molecules. Very soon, however, he was compelled to alter his view.

For the intellectual movement he helped to lead had scarcely begun when evidence in plenty for the objective existence of molecules came to hand and the atom became for everyone a reality, not indeed as the indivisible atom of Dalton but as a system with such properties that the mind is automatically compelled to attempt its visualisation. So real is our knowledge of actual molecules to-day that we have accurate information as to their dimensions, while the number contained in a given mass of any pure substance has been determined by various methods giving extraordinarily consistent results. The work of Perrin, Millikan and others has shown that a gram molecule of a substance contains  $6.05 \times 10^{23}$  molecules, the probable error in this figure being at most exceedingly small. Moreover, the structural formulae which the organic chemist has ascribed to complex molecules can no longer be looked upon by anybody as mere conventions, summarising the behaviour of substances conveniently and consistently, but essentially unreal. The brilliant researches initiated by Hardy and Langmuir and carried on by N. K. Adam and others, dealing with surface films one molecule in thickness, which are formed by the spread of certain substances upon an air-water interface have yielded surprisingly conclusive

evidence for the details of molecular form and have yielded measurements of individual localised parts of the molecule concerned, of the length of a particular carbon chain, of the diameter of a carboxyl group, and so on.

I have thought it worth while to remind you here of this brief non-atomistic interlude in scientific thought and the fresh readjustments to kinetics and to the mechanistic outlook which have followed upon the remarkable developments of recent years, because the circumstances bear upon the present relation of biology to the more exact sciences. It is not too much I think to say that these developments have for a time at least imposed upon the mind of the experimental physicist and the organic chemist a mode of thought which is essential for the biologist—the *visualisation of a mechanism*. Biology has always had to relate the behaviour of the objects it studies to the properties of highly complex systems considered as a whole; whereas the exact sciences long progressed owing to the fact that their material allowed them to reduce most, or many, of the problems to such simple terms that the essential significance of the data remained independent of underlying mechanism which needs visualisation. On such lines thermodynamics developed with its complete independence of mechanistic considerations. This independence is the essence of the power and the attractiveness of its methods, but that power is strangely lessened with phenomena which remain under conditions of which the very essence is complexity. Complexity is the essence of the material systems which manifest the phenomena of life; it cannot be eliminated from the only kind of picture of such systems which is real.

Meanwhile, at any rate, the physicist visualises an atom which, no less than the living cell, is a microcosm, a mechanism of which the totality is something more than the sum of its parts; and the organic chemist visualises with confidence his molecules as real systems, and, be it noted, no longer as stable structures but as systems which, like the atom on the one hand and the living cell on the other, maintain their essential identity although a seat of continual change. Like living organisms they are not "stable" but "stationary" in form. These analogies may be remote, but they are by no means unreal. They are real in respect of fundamental concepts and categories.

Certain modern thinkers indeed have recognised them as being real enough to take their place among more general considerations which, duly appraised, seem to call for a recasting of scientific views concerning reality in the universe as a whole. Professor Johann Hjort, the Norwegian zoologist and oceanographer, foreshadowed such a view in his *Unity of Science*, a book published six years ago. Dr. A. N. Whitehead, in his *Lowell Lectures in 1925*, since published in a book entitled *Science and the Modern World*, has followed the ideas involved to very profound conclusions. For those who have not read this highly important work it is impossible to convey any adequate idea of its argument in a few paragraphs; but certain aspects of Dr. Whitehead's thought bear so directly upon what I myself have been

trying to express that I shall do well to refer to them. The conception of all that is implied in the term "organism" is for him fundamental. He shows that the conception derives from biological thought, but holds that it underlies all concrete reality. Dismissing all forms of vitalism as representing a mere compromise, and feeling that "the gap between living and dead matter is too vague and problematical to bear the weight of such an arbitrary assumption as vitalism involves," he holds, on the other hand, that *all* concrete enduring entities are organisms, and suggests that biological science, which has always had to face what is implied in the term, "is only now coming to a growth adequate to impress its conceptions upon philosophy." "The relation of part to whole," he writes, "has the special reciprocity associated with the notion of organism in which the part is for the whole; but this relation reigns throughout nature and does not start with the special case of the higher organisms." Science, indeed, "is taking on a new aspect which is neither purely physical nor purely biological. It is becoming the study of organisms. Biology is the study of the larger organisms; whereas physics is the study of the smaller organisms." He calls his own standpoint one of "organic mechanism." I cannot attempt here even to indicate the manifold arguments which give reality to these conclusions; they cannot be put shortly and they require much concentration of thought. In a sentence we may say that for Dr. Whitehead concrete reality only emerges when entities usually made abstract in scientific thought display relatedness. I will only add that nowhere does he suggest that it is illogical to attempt analysis of an organism. In respect of the living organisms he seems rather to encourage the chemist. We may, I think, admit that the physicist and the chemist would look upon any suggestion that they should abstain from analysis of the organisms they deal with as highly illogical.

I would now like to return to a consideration of Dr. J. S. Haldane's position. The professed biochemist has no right to ignore the intellectual standpoint of one to whom his science owes so much. For Dr. Haldane, though he will certainly not admit that his brilliant researches have ever been directed towards the analysis of living organisms into chemical and physical mechanisms, has provided admirable methods and important data for biochemistry however its task may be defined.

His physiological teaching is doubtless based upon his general philosophical outlook, though it is not for me to attempt to show how one is derived from the other. My aim is only to discover whether his attitude as a physiologist should necessarily disturb the mind of the biochemist, defining the latter as one who from the nature of his work and training must think about the organism in terms of chemical mechanisms.

It is of great interest to note that in the very latest exposition of his views (published while this lecture was being written) Dr. Haldane has shown that he himself has felt the significance of the circumstance that recent developments have brought the biologist and the physicist or chemist nearer

together in respect of their modes of thought; he says, "the new physical conceptions of matter allied in certain fundamental respects to those which I have tried to explain, that the latter challenge to them, but furnishes hints and suggestions while, conversely, the new physical conceptions of matter challenge to them, but furnishes hints and suggestions. In other words, physics and biology are not so far apart as of reducing biology to the status of a branch of physics, but on the lines of so fundamentally modifying physics and chemistry that a meeting point between the two can be anticipated." Yet the general conclusion which these words appear is still that there is a fundamental difference between biology and the physical sciences and that we must keep them separate.

The remark I have just quoted may have been intended to apply the doctrine of relativity upon scientific thought; but it must incidentally refer to the circumstances of life with atoms which are organisms.

The atom, we now find, is neither a pure unit, but a mechanism or organism in which the whole depends not solely on the nature of its parts but on the relations of its parts; while an organic molecule is neither a pure unit, but a mechanism or organism in which the whole depends upon the relatedness of its parts. Now for *descriptive* purposes, and for visual representation, the atom or molecule must be in our minds; and we must accept the results of analysis. Yet in spite of such fundamental properties of the *whole* will elude us if we try to grasp it while intact. We can only properly define the properties of the activities. Remember that neither the atom nor the molecule is a system. The *behaviour* of each depends not only upon the nature of events which occur, but also upon the nature of events which occur in the environment to ascertain in each case how the atom or molecule behaves; just as we have to in the case of what we call an organism. In all cases structure, internal organization, and thought of as inseparable if we are to grasp the nature of the systems; but as every one will admit (in the case of the atom at least) we could hardly obtain that grasp if we try to grasp the parts to subserve our mental synthesis. It is, therefore, while, to show how the atom and the organism are, in a sense, at one with their environment, a circular argument in the case of the living organism. I will refrain however from such analogies. I may be already in danger of overdoing it that I have strained them overmuch. It is not the atom or the molecule to complex systems which

of myriads of such units; but I am convinced that the considerations I have put before you are significant. We are dealing with a very fundamental matter—the question of *categories*. The contention of the organicist seems to be that the living organism as a system is in an entirely different category from any system studied by the physicist or chemist; but the criteria upon which he relies are surely applicable to all systems which are at once dynamic and enduring. Such are the atom and the molecule as we visualise them to-day. While recognising many limitations to the statement, we are entitled to say with Dr. Whitehead that the “organisms” studied by the biologist are made up of the “organisms” studied by the physicist or chemist.

The aim of modern biochemistry, however, is not merely to analyse the former organisms into the smaller ones which compose them. This would be only to arrive at the substances present, a superficial if necessary task. The analysis in question involves the study of such factors or mechanisms as the properties of surfaces within the organism; the nature and course of individual reactions which occur in it; the nature of its catalysts, its equilibrium relations, and the like. This is an analysis carried out with the hope of some final intellectual synthesis amounting to a real description. If now we recall the pronouncement of Dr. Haldane from which this discussion started, and consider his view (not likely to be affected I imagine by our dealings with categories!) that the above sort of analysis can only end in colossal failure, it may be that some misunderstanding concerning the meaning of words must be first avoided. The term mechanism may have various meanings, as we have already seen, and it is well to be sure as to what “failure” means. If by chance it means failure to “explain,” then it must be admitted that the biochemist does not hope to explain; his humbler aim is to describe. To be as sure as possible of what precisely Dr. Haldane means we must seek for points in his teaching which are concrete. To the question, what (when forbidden to analyse the organism into simpler mechanism) is the precise task of the investigator; he has many times supplied an answer. One form it has taken is the following: the physiologist is to “seek for interconnected normals, and their organisation with reference to one another and to other organic normals.” This seems to mean that he is to illustrate by observation how wonderfully the body is adjusted, but to ignore the means of adjustment, so far as they are physical or chemical. It almost implies that, starting with the preconceived idea of a perfectly adjusted organism, emphasising in particular its ability to return to a normal after any disturbance, he is merely to consolidate his faith by repeated demonstration of its perfection. This may not be a statement which is wholly fair, but I hold that there is an a-prioristic element in such a point of view which may misdirect experimental studies.

Somebody has remarked (I think Wilhelm Ostwald, though I quote from memory) that between the mental employment of Platonic “ideas” and the mathematical formulation of “natural laws” there is much in common and both may inhibit experimental advances. Even to-day, in spite of the

intellectual revolution in science, there is to be found the tendency to see in a so-called natural law something above appearances, something which appearances *ought* to obey. Once a so-called law of nature has got itself formulated, there are few who can avoid feeling that experimental results which conform to it are likely to be more accurate than those which do not; whereas it may well be that the "law" is inexact, and not the incongruous data. This assuredly has been dramatically illustrated by the [extension] of Newtonian [physics by relativistic Einsteinian physics].

But great as the danger of over-sublimating "laws" is from the standpoint of experimental biology, that of starting with a sublimated idea of an organism is just as great. The idea is that of a perfectly autonomous system, capable of continuous adaptation without loss of individuality; highly unstable, yet in form and essence stationary; in control of its environment external and internal; tending ever, after disturbance, to return to a fixed normal. To this ideal actual organisms doubtless approximate and some more than others. The human body's *vis medicatrix naturae* [is a case in point], but the adjustment is [not] perfect. Evolution must have left behind many failures due to imperfect adjustment; yet these failures were organisms while they survived. It should not be forgotten that where there is failure to reach this ideal the facts concerning this failure are just as significant as those which demonstrate a nearer attainment. Be it remarked moreover that the experimentalist who begins his study of an organism with a preconceived conception of the kind is definitely biased in favour of data which seem to conform to it, and against those which do not; he is always more interested in the former.

The complete adaptation of living organisms is a hypothesis which should be tested in every case. A bias in favour of "organicism" may lead to misinterpretation of experimental data just as readily as the bias in favour of too facile "mechanism" which ruled in the later years of the last century.

If we are yet a little vague as to what are the mechanisms in terms of which an organism (we will here think of the human body in particular) is *not* to be analysed, another concrete illustration will assist us. I will select a simple case. Largely as the result of pioneer studies by Dr. J. S. Haldane and his co-workers we know that the body is admirably adjusted to maintain with extreme constancy the chemical reaction, or the hydrogen ion concentration, of the blood; an essential quality for a normal internal environment of the tissue cells. The interest of this circumstance has led numerous experimentalists, led by Dr. L. J. Henderson, to study the chemical and physico-chemical factors involved in the adjustment. Very accurate data concerning the acid-base equilibria have been obtained, and among many other details has emerged the importance of the so-called buffer effect due to the influence of the bicarbonates and certain proteins in blood exerted on lines which I assume I need not stop to explain. In respect of these chemical facts Dr. Haldane, deprecating the search for "mechanism," writes as follows: "It is common, for instance, to speak of the regulation of reaction

in the living body as if this were an equilibrium of acid and base, maintained approximately steady by the presence in the body of buffering substances, *instead of by physiological activity*" (the italics are mine). "Language of this sort" (he continues) "is imagined to be scientific: it seems to me only evidence of lack of scientific insight." I have striven hard to meet his thought here with little success. This buffering action is, of course, only one of the factors concerned in the regulation, and all are co-ordinated "physiologically"; but does this circumstance lessen the extreme significance of the simple objective nature of this particular factor or mechanism? If "physiological activity" were a form of activity in which the buffer action of salts were really of no account, if this action only emerges into apparent significance because artificially isolated from the physiological whole, then some understanding of the position taken up might be possible. But the fact that the blood is a buffered system is a datum for physiology as real as the more embracing fact that the blood reaction is kept constant. As my colleague, Dr. Joseph Needham, has claimed, the recognition of this simple phenomenon of buffering in the body gives us a key position from which one aspect of many phenomena can be seen. In recognising it as a mechanism of adjustment characteristic of the body we need not forget that its full significance only emerges when it is correlated with everything else that is characteristic of the body.

The faith and the scepticism of the organicist alike limit the domain of experiment which, if he be consistent, is open to him. If he be quite faithful to his teaching, it is only on the intact organism that his observations can be made. Dr. Haldane has himself largely accepted this limitation and, such is his special genius, has, in spite of it, put physiology very deeply in his debt. Yet it is sure that physiology as a whole would have suffered grievously if all investigators had admitted the necessity of such a limitation. It is true, of course, that there is important knowledge which can only be obtained from experiments on the intact, or nearly intact, organism. This is very limited in its scope, however, and standing alone would be far indeed from yielding us even our present ability to visualise the organism as a whole. From countless possible illustrations of this statement, which I think will seem to most a truism, I will choose only one; and since I am dealing with biochemistry I will suggest a chemical illustration.

What can be more characteristic of the body as a whole than the circumstance that harmony among its parts is secured, not alone by the functions of the nervous system, but by the circulation of definite chemical substances—the hormones. An infinite number of observations upon the intact body would have failed to reveal the existence of these. We could have known nothing about them had we not set about the task of analysing the body into chemical mechanisms.

I think at this point we may try to come to grips with what is truly definite about these antagonisms in thought which you have been asked to consider. We need some crucial test as to how matters really stand; because

there is, after all, something elusive about the attitude of the organicist. Even at this moment I am myself not perfectly clear, for instance, whether Dr. Haldane, when he made that sweeping statement quoted at the beginning of this lecture about colossal failure, meant a failure in thought or a failure in method. I think the organicist should answer such frank questions as the following: The buffering power which we can so readily observe in drawn blood, is it not a property displayed by normal blood when circulating in the intact body? The effects we observe when a preparation of a hormone is injected into an animal, do they not occur when, in the intact animal, the hormone reaches the blood by normal channels? Or, *as the only alternative, are these properties of the blood and of the hormone created by the observer's methods? Is there in the intact organism some mystical whole from which such properties of parts never even emerge?* If he hold to the latter alternative, the organicist maintains a logical position, but he will, I fancy, have fewer disciples than has the frank vitalist, if only because his gospel is so very hard to understand. If he allow the former alternative, then how can he ascribe colossal failure to all efforts to analyse the organism into chemical mechanisms? Without the information they impart, knowledge and grasp of the organism as a whole can at best be superficial and very incomplete. If there be any lack of reality about the knowledge won by such efforts, it only emerges, I think, in those subtle workings of the philosophic mind which see reality disappear during every process of analysis. Experimental science has always been face to face with a philosophic caveat of this kind, but the chemist dealing with living organisms has no more to fear from it than any other experimentalist. There are aspects of the organism which must be left to the philosopher to deal with, if he can. But surely the soundest philosophy is that which, instead of ignoring any of the hard won data of experimental science, should use them all—each in its place—in his endeavour to arrive as nearly as possible at reality.

N.B.—Some small additions to the text of this lecture had to be supplied for lacunae in the MS. by the Editors; these are indicated by square brackets.

## THE EARLIER HISTORY OF VITAMIN RESEARCH

[Nobel Lecture delivered on 11th December, 1929]

WHEN the present century began, animal nutrition was being viewed too exclusively from the standpoint of energy requirements. The fundamental pioneer work of Rubner and its later extension to human subjects in the remarkable enterprise of Atwater, Benedict, Rosa and others in the United States could not fail to produce a deep impression upon the thought of the time. The quantitative character of the data obtained and the attractive circumstance that such data appeared to supply a real measure of nutritional needs, independent of, and apparently superior to, considerations based upon chemical details, induced a feeling that knowledge concerning these needs had become highly adequate and was approximating even to finality. As a matter of fact, however, these calorimetric studies, invaluable in themselves, were then leading to doctrinal teaching which contained inherent errors. So fundamental an aspect of the then dominant doctrine as, for instance, the law of isodynamic equivalence among food-stuffs, is at the most approximately true, and fails entirely when the equivalence is tested by physiological results rather than by purely physical data. The assumption indeed that carbohydrates and fats can replace each other indefinitely in a diet, so long as the total energy supplied remains the same, has led to serious errors in practical dietetics. More serious in leading to error was the assumption that all proteins were of equal nutritive value, and most serious of all in this respect was, as we now know, the belief that proteins, carbohydrate, fat and suitable inorganic materials supplied in themselves all the needs of the organism. Inhibitory, moreover, was the odd assumption often to be detected in the writings of leading authorities that to view nutritional needs from the standpoint of energetics was not alone more convenient, but more scientific, and even more philosophical, than to discuss them in terms of the material supply.

It must, of course, be most fully recognised that the calorimetric studies so dominant twenty years ago and, no less, the quantitative studies of respiratory exchange which aided and extended them, provided knowledge which is of the utmost importance and a permanent asset of science. The prime demand of the active organism is for energy, and when all the more specific demands are met in a well-balanced food supply, the available energy becomes, of course, the limiting factor which determines the adequacy of that supply. The studies in question were, moreover, usually made upon subjects consuming natural foods and the diet was therefore, in general, sufficiently well balanced. When it was not, the inevitable brevity

of calorimetric observations failed to reveal effects of dietetic deficiencies which require time for their display. Such studies have therefore retained their value, and will yet doubtless be extended: but had they continued to dominate the whole field of activity, had they not been supplemented by a more discriminative inquiry into the various factors which determine real adequacy in nutrition, our knowledge would have remained highly imperfect, and our views erroneous. For now that the organic material factors present in dietaries actually capable of maintaining normal nutrition for long periods have been more carefully explored, it has become evident that, so far from comprising protein, fat and carbohydrate alone, they are many and diverse, and each is indispensable. We have even come to know that a diet which, apparently at least, will support the individual throughout life may yet lack a factor which is necessary to maintain an adequate capacity for parentage, so the diversity of materials indispensable for normality is seen to be yet greater. The complexity of these nutritional needs as we now view them is indeed astonishing. We find them displayed in the details of the protein supply, in the call for a right balance among the inorganic constituents of a diet, and, particularly, in the urgent call of the body for a number of organic substances specific in nature and function, in respect of which, however, the quantitative supply is, in accordance with the demand, so small as to contribute little or nothing to the energy factor in nutrition. These substances, following the suggestion of Casimir Funk, we have agreed to call vitamins.

Who was the "discoverer" of vitamins? This question has no clear answer. So often in the development of science a fundamental idea is foreshadowed in many quarters, but has long to wait before it emerges as a basis of accepted knowledge. As in other cases, so with recognition of vitamins as physiological necessities. Their existence was foreshadowed long ago, but a certain right moment in the history of the science of nutrition had to arrive before it could attain to universal recognition. Some workers had discovered suggestive facts, but failed to realise their full significance. On the other hand, the work and words of true pioneers lay forgotten because published when average minds were not ready to appraise them at their right value.

Some fifteen or sixteen years ago the importance of vitamins became somewhat suddenly recognised. So enormous now is the existing literature concerning them, so complex and, sometimes, so uncertain, are the issues raised, that it is impossible to survey the subject adequately in a single lecture. The circumstances of my most enviable position here to-day will justify me in dealing rather with the earlier history of the subject, and I will venture in virtue of that position to put before you certain personal experiences which have no place in the proper history of the subject. They have not been, and will not be, published elsewhere.

No one can deny that the recorded experience of voyagers and explorers in the eighteenth century, and particularly perhaps the records of the



the challenge. Perhaps an explanation of this is to be found in the circumstances that Bunge, though in his well-known book he remarks that it would be worth while to continue the experiments which had suggested the existence of unknown nutritional factors, was, as I happen to know, himself inclined to disbelieve in them. He thought that the real error in the synthetic diets used by his pupils (which was, so to speak, "dissected milk") was that the method of its preparation had involved the separation of inorganic constituents from certain organic combinations in which latter form alone could they adequately subserve the purposes of metabolism. Other causes may have contributed to the long neglect, first of Lunin's and then of Socin's suggestions, and it must be admitted indeed that no *experimentum crucis* was carried out in Bunge's laboratory.

In Lunin's experiments the fate of six mice only (those placed by him upon a normal salt mixture) really suggested the existence of unknown factors; and no data are given as to their consumption of food. Neither Lunin nor Socin made any attempt to complete the evidence by making discriminative additions to the diets which had proved inadequate. Lastly, as I have already suggested, since the main intention of their work, and the titles of their publications, were remote from the special issue, their significant remarks might well appear as mere "*obiter dicta*" when read without the light of modern developments.

Yet the pregnant suggestions arising from the observations just discussed did ultimately, though not for fourteen years after the latest of them were published, awaken (as we may suppose) the interest of an investigator distinguished in many fields, who was led to repeat and extend them. I allude to the late Professor Pekelharing, whose own observations (published in 1905) unhappily again remained unknown to the majority till very recently. It is indeed astonishing that the results of such significant work as his, though published in the Dutch language alone, should not have become rapidly broadcast. I cannot refrain from referring to the circumstance that the paper was not abstracted or mentioned in Maly's *Jahresbericht für Tierchemie*, so adequate, and in general so complete, in its dealings with current literature. Many of us were accustomed to rely upon it for references to work published in journals that we could not consult, or in a language that we could not read. Though other work by Pekelharing was duly recorded at this time, no mention was made of the extraordinarily interesting paper in question. My own experiments began soon after the paper was published, and as a proportion of my own work was very similar to that of Pekelharing, I shall never cease to regret that, in common with so many others, I was then completely ignorant of the latter. After speaking of experiments carried out on lines similar to some of those done in Bunge's laboratory and indicating that they pointed to the existence of some unknown essential, Pekelharing goes on to say: "Till now my efforts, constantly repeated during the last few years, to separate this substance and get to know more about it have not led to a satisfactory result, so I shall not



a category that their indications might be neglected when the needs of normal nutrition were being estimated.

No general or widespread belief in the view that an adequate diet must contain indispensable constituents other than adequate calories, a minimum of protein, and a proper mineral supply, could be said to exist till the years 1911-12. Those years saw the appearance of my own publications. Thereafter started a period of great activity in the study of the facts; immediately in the United States of America, a little later, and still more after the war, in many centres.

I will now refer to my own experiments, and will also intrude upon your patience with a reference to those personal experiences of which I spoke at the beginning of this address.

Early in my career I became convinced that current teaching concerning nutrition was inadequate, and while still a student in hospital in the earlier eighteen-nineties I made up my mind that the part played by nutritional errors in the causation of disease was underrated. The current treatment of scurvy and rickets seemed to me to ignore the significance of the old recorded observations. I had then a great ambition to study those diseases from a nutritional standpoint; but fate decreed that I was to lose contact with clinical material. I had to employ myself in the laboratory on more academic lines. I realised, however, as did many others at the last century's close, that for a full understanding of nutrition, no less than for an understanding of so many other aspects of biochemistry, further knowledge of proteins was then a prerequisite; and when I was first called to the University of Cambridge I did my best to contribute to that knowledge.

As an ultimate outcome of my experiments dealing with the relative metabolic importance of individual amino-acids from protein my attention was inevitably turned, without, I think, knowledge, or at any rate without memory, of the earlier work, to the necessity for supplying other factors than the then recognised basal elements of diet if the growth and health of an animal were to be maintained. This indeed must at any time come home to every observer who employs in feeding experiments a synthetic dietary composed of adequately purified materials. It was the experience of the workers in Bunge's laboratory long ago; it was, as we have lately learned, the experience of Pekelharing. A good many investigators using synthetic dietaries have, it is true, from time to time expressed doubts upon the point, but we now know that it was because the constituents they used were not pure and not free from adherent vitamins. In 1906-7 I convinced myself by experiments, carried out, as were those of Lunin and Socin, upon mice, that those small animals at any rate could not survive upon a mixture of the basal foodstuffs alone. I was especially struck at this time, I remember, by striking differences in the apparent nutritive value of different supplies of casein in my possession. One sample used as a protein supply in a synthetic dietary might support moderate growth, while another failed even to maintain the animals. I found that a sample

of the former sort, if thoroughly washed with water and alcohol, lost its power of support, while addition of the very small amount of extract restored this power and also, if added to the samples originally inadequate, made them to some degree efficient in maintaining growth. I found further at that time (1906-7) that small amounts of a yeast extract were more efficient than the casein extracts. Similar experiences were encountered when otherwise adequate mixtures of amino-acids were used to replace intact proteins. By sheer good fortune, as it afterwards turned out, I used butter as a fat supply in these early experiments. Upon the evidence of these earlier results I made a public statement in 1907 which has been often quoted. I cannot, however, justly base any claims for any sort of priority upon it, as my experimental evidence was not given on that occasion. It was indeed not till four years later that I published any experimental data. In explanation of this delay I would ask you to consider the circumstances of the time. The early experiments of Lunin and others had been forgotten by most; the calorimetric studies held the field and tentative suggestions concerning their inadequacy were, I found, received with hesitation among my physiological acquaintances. It seemed that a somewhat rigid proof of the facts would be necessary before publication was desirable. Thus came the great temptation to endeavour to isolate the active substance or substances before publication, and I can claim that throughout the year 1909 I was engaged upon such attempts, though without success. At this time I was using what is now the classic subject for vitamin studies, namely the rat. As I was concerned with the maintenance of growth in the animal, the tests applied to successive products of a fractionation took much longer than those which could be used in studying the cure of polyneuritis in birds by what we have learned to call vitamin B<sub>1</sub>, so the work occupied much time. I may perhaps be allowed to mention what was for me a somewhat unfortunate happening in the beginning of 1910, as it is instructive. A commercial firm had prepared for me a special extract of a very large quantity of yeast made on lines that I had found effective on a small scale. With this I intended to repeat some fractionations which had appeared promising. I thought, however, upon trial that the whole product was inactive and it was thrown away. The real explanation, however, was that instead of using butter, as in earlier experiments, I at this moment determined to use lard, and my supply of this, as I learned to understand much later, was doubtless deficient in vitamins A and D; I was now giving my animals in the main the B group alone. If I had then had the acumen to suspect that any of the substances I was seeking might be associated with fat I should have progressed faster. Later in 1910, if I may intrude so personal a matter, I suffered a severe breakdown in health and could do nothing further during the year. On my return to work I felt that the evidence I had by then accumulated would be greatly strengthened by a study of the energy consumption of rats, on the one hand when failing on diets free from the accessory factors (as I had then come

to call them), and, on the other hand, when, as the result of the addition of minute quantities of milk, they were growing vigorously. These experiments took a long time, but they showed conclusively, as at that time it seemed necessary to show, that the failure in the former group of animals was not due primarily, or at the outset of the feeding, to any deficiency in the total uptake of food.

My 1912 paper is sometimes unfairly quoted as though its bearing applied only to the influence of minimal quantities of milk upon nutrition. It will be found, however, that it emphasises on general lines the indispensable nature of food constituents which were then receiving no serious consideration as physiological necessities.

In a personal endeavour to estimate the influence of my publications in 1912 upon the opinion of the time, and their relative importance in the initiation of that great activity in kindred studies which shortly followed their appearance, I have found it necessary to consider at the same time and particularly the work and writings of Casimir Funk. It is sure that, until the period 1911-12, the earlier suggestions in the literature pointing to the existence of vitamins lay buried. There is no evidence, I think, that they were affecting the orientation of any authoritative teaching concerning the phenomena of normal nutrition either at the time in question or indeed, in any effective sense, before.

A few years ago in the American journal *Science* Funk published a short article in which, after giving me some credit for prophetic vision, he protests against my being called the "discoverer of vitamins." In this protest he was justified; I have certainly never made any personal claim to be their "discoverer," and all the past circumstances of which I have reminded you have deprived perhaps every individual worker of that clear title. Funk, however, further remarks in the article mentioned that my chief paper appeared too late to affect the situation to any appreciable degree; a remark which I believe to be entirely unjust.

F. Röhmman, an experienced worker on nutritional problems, and much concerned with the chemical side of them, but one who never fully believed in the claims made for vitamins, wrote in 1916 after discussing the earlier literature, "Als der geistige Vater der Vitaminlehre ist wohl Gowland Hopkins zu betrachten, während die Bezeichnung Vitamine von Casimir Funk her stammt." Such a statement without extension does of course far less than justice to Funk's influence, which in many ways was important. Funk himself, however, made no experiments which bore upon the physiological functions of vitamins until long after my paper had appeared.

Funk's first entry into kindred fields was in a paper published in December, 1911, describing his earlier efforts to isolate the curative substance from rice polishings. He continued this effort, and further papers appeared in 1912, when Suzuki and others were also describing their endeavours to isolate the substance. Funk's attempt was extremely praiseworthy and his publications doubtless awakened new interest in Eijkman's original

discovery. He did not, however, succeed in isolating any substance which has since been accepted as being in fact any actual vitamin of the B group, and the papers in question contain no suggestion bearing on the general physiological importance of vitamins.

In June, 1912, however, Funk published a paper dealing with the "Etiology of Deficiency Diseases," which was a valuable summary of the existing knowledge concerning such conditions, and here he showed more clearly than had been shown before how definite a group is constituted by these diseases. He also emphasised in an interesting way and in advance of general opinion the view that pellagra would prove to be one of them. This review contains, however, no account of personal work other than the attempts already mentioned to isolate the anti-beriberi factor. A short final section purports to relate the available knowledge concerning deficiency diseases to the facts of normal metabolism and nutrition. I feel justified in saying that this section is written in a manner which is essentially disingenuous. The author says, for instance, "I suppose that the substance facilitating growth found in milk is similar to, if not identical with, the 'vitamins' described by me." But Funk had then "described" no vitamins. He had in this review—the merits of which I have already emphasised—on theoretical grounds and with reliance mainly on the work of others—only made the doubtless very significant suggestion that each deficiency disease might depend upon the absence of its own specific factor. He admits knowledge of the existence of my experimental results; knowledge obtained—according to a footnote—through a "private communication." In the article in *Science* to which I have earlier referred, Funk states, on the other hand, that he had in 1912 no knowledge of my work. I am entitled to say that early in October, 1911, I gave a very full account of my results at a meeting of the English Biochemical Club, and I certainly obtained the impression during the succeeding months that they had become well-known to English physiologists and biochemists generally.\*

In closing these references to Casimir Funk's writings and to his earlier work I would like to make clear my belief that he has not received too much, but too little, credit for his vitamin work as a whole. I venture to think, however, that he was in no sense my predecessor in the physiological field. I may say that till now I have had no intention of commenting on his remarks in the *Science* article of 1925. Only the peculiar circumstances of my present position, which seem incompatible with his view, have led me to discuss it.

Very soon after my chief paper appeared the study of vitamins was,

\* On 28th February, and again on 8th March, 1911, the London *Daily Mail* gave great publicity to certain statements of my own which, though distorted for journalistic purposes, made essentially clear the conception of vitamins as based upon my personal experiments. These articles were quoted in the Continental, and with especial freedom, in the American press. At the time I much regretted this unsought publicity. My main paper, the publication of which was much delayed in the press, appeared in the month following Funk's review.

as you know, developed with great energy and success in the United States. We owe especially to Osborne and Mendel, and to McCollum and his co-workers, the all-important work which continued during the earlier years of the war. The proof on the part of the American authors of the distinction between what were then known as the "water soluble" and "fat soluble" vitamins was the first clear evidence of diversity among the vitamin factors required for growth. This discovery made all later studies more discriminating, and the pioneer work of the authors mentioned was of the greatest importance. So prominent indeed was the American work at this time and so large a proportion did it form of the total output from 1912 to near the end of the war that, if I wished to claim that my own publications exerted any real and effective influence in starting a new movement in the study of dietetics, I should have to convince myself that they helped to direct the thoughts of the Harvard and Baltimore investigators. Anyone reading with care the succession of papers describing their experimental studies before and after the appearance of my own publications in 1912 will, I think, become convinced that such directive influence was indeed exerted. This circumstance and much correspondence received at the time from European colleagues made me feel then that my paper had served the purpose I had wished for it, namely, to direct thought concerning normal nutrition into a channel which, if not new, had been long and strangely neglected. For a time indeed I thought that channel to be even new. I was at least a pioneer whose efforts were not wasted, and I am always now content to recall an opinion expressed by the late Franz Hofmeister, the most just, if also the most generous of critics. Hofmeister, in 1918, after an exhaustive study of the whole literature, speaks of me as the first to realise the full significance of the facts. If that be true, and if, as well may be, that has been the view of the Nobel Commissioners who have thought me worthy of so great a reward, I can happily enjoy my good fortune.

## ADDRESS UPON AN UNKNOWN OCCASION

(ca. 1930)

It is my faith, which must be shared I think by all who are versed in the history of science and industry, that every generous encouragement given to the advancement of chemistry in this country, benefits not local interests alone, but the nation. Increased opportunities, wherever given, for teaching and research in the subject, increase proportionately the possibility of discoveries which become of universal benefit. Chemistry, of course, is one of the basal sciences, and on its progress advances in very many other branches of natural knowledge depend, and this in increasing measure to-day. The aid it has given in the past to the sister science of physics is to-day being handsomely repaid; but the thought of the physicist is still influenced by the thought of the chemist, and though they are now drawing closer together, there is still a real distinction inherent in their respective modes of thought which results in material benefit.

To-day we are learning how important is chemistry to all biological sciences, pure and applied. To clinical medicine its services are rapidly becoming more and more real, not only on such lines as those of chemotherapy so-called—the production of valuable synthetic drugs—but in aiding diagnosis and in revealing aspects of disease which would remain obscure but for the application of chemical methods of study. It is a striking circumstance that even the psychiatrist is now turning to chemists for their help. Many forms of mental disorders would seem to depend on disturbance of metabolism which are revealed by chemical studies, and there is increasing hope that methods of treatment may be found to correct the errors so discovered. If some forms only of insanity can be cured it will be at any rate a partial solution of one of the most painful of human problems. Extending this, may we not believe that even the psychologist may have to give some thought to chemistry. So intimately interwoven are the activities of mind and body that no one can deny that the psychology of the individual is greatly affected by the hormonal balance which is part of his physical constitution, and hormones owe their specific influence in the body to particular aspects of their molecular structure. I have, however, indulged in these latest references only to illustrate how ubiquitous the services of chemistry may be. To its current and more immediate services to biological science I will make further reference later.

It is hardly necessary for me to emphasise here the outstanding services which chemistry has rendered and is always rendering to industry. It is very many years since he, who afterwards became Lord Beaconsfield, in a noteworthy speech as Chancellor of the Exchequer asserted that the prosperity of the industries was a reliable index of the prosperity of the country

as a whole. The British public and its Government realised the significance of this claim very slowly; indeed it needed the lessons of the war to bring it fully home to them, but it is well understood to-day. On the services of chemistry to national defence we must touch but gently, deep and diverse feelings are here involved. One thing is sure: those who believe in the necessity of an increase in our defences must recognise that to-day the services of the chemist are all-important to their adequacy. It might be tempting to illustrate by examples the influence of chemical advance on the progress of all the greater among our current industries, but the illustrations would be so numerous that time would not permit. They are familiar to most, and industrial applications are outside the main intention of my address.

There are many kinds of chemist, each kind entering the field of endeavour by a path of its own; but all chemists are brothers under the skin. Roughly, I suppose, they may be classed under the headings: physical chemists, organic chemists, and to-day, if you will accept the distinction, biochemists. The work of each one of them serves pure science and applied science alike. The distinction between the pure and the applied is indeed becoming daily less real.

Circumstances make me wish to refer more particularly to the work of the organic chemist, and to the development of classical organic chemistry as an accomplishment of the human mind, which is unique in kind. A few years ago, amid the intellectual excitement caused by the rapid developments in atomic physics, I often heard it said that, intellectually, organic chemistry had reached a condition of stasis; that little was left for it but to go on making new compounds on well established technical lines. I believe that, on the contrary, never did it show more promise of continued intellectual progress than at this moment.

I would like, however, to make a brief reference to its past. The interested but uninstructed layman to-day seems often to follow (or to endeavour to follow) many of the less technical and more exciting developments in modern atomic physics, and by reading able and highly popular books comes to indulge pleasantly, if not always adequately, in the quasi-philosophical speculations which mathematical physics has engendered. On the other hand, I have always found that there are comparatively few among the intelligent lay public who can properly appraise the intellectual accomplishment involved in determining and reproducing the structure of molecules—in dealing, that is, with the actual architecture of entities for ever invisible, except to the eye of the mind.

I am myself far enough removed from being a proficient or expert organic chemist to be able to speak without bias, and I always find pleasure in pointing out to the layman who has never thought about it, and even sometimes to the young chemist who takes it for granted, how remarkable *in kind* was the intellectual progress of organic chemistry during its classical period, say from the time of Liebig and Wöhler through that of Kekule to that of

Emil Fischer. I fear I may, in my time, have indulged too often in expounding this theme in public. Here I must be content with a brief reference to it.

Many of you may remember that the philosopher Kant, in an attempt to decide what aspects of human knowledge should come into the category of sciences, decided that only those susceptible of being put into mathematical form should be thus honoured. Even so, he himself seems to have been disconcerted by finding that such a definition would exclude the chemistry of his day. Had he lived to see the development of organic chemistry during the last half of the nineteenth century, he could scarcely have failed to widen his definition of a science. The analytical methods of classical organic chemistry were, of course, necessarily quantitative, but it cannot be claimed that the intellectual processes which led to the conception of molecular structure were in any sense mathematical. They were rather pictorial and almost artistic in kind. They involved, it is true, the right interpretation and the logical application of evidence of a sort which was at first quite new to the mind, but the triumphant application of that evidence leading to the grasp of molecular architecture and the almost magical constructive powers based upon that grasp, surely involved vivid pictorial thought and trained intuition unserved by mathematics. Pictorial thought in science is often held to be inferior to mathematical thought, and in many fields this is doubtless true, but in this particular field, because of its very nature, the former triumphed. Its true accomplishment indeed has only been fully revealed of late. Many even among the chemists themselves were apt to look upon their structural formulae as being merely useful and ingenious diagrammatic summaries of the facts known about the substances represented, and not real in any acceptable sense. But, as you know, modern technique has shown that the accepted formula of organic chemistry approaches much nearer to reality than this. The carbon ring, the carbon chain, the polar group, these are not merely convenient and useful fictions, they are part of whatever aspects of reality it is in the power of science to reveal.

Such was the nature of the knowledge won by the methods and modes of thought of classical organic chemistry. There is still much to be done by those who use them, and it is to be hoped that the intellectual equipment they demand will not be lost by a generation which is rejoicing in new and fascinating experimental methods by which still subtler details of molecular structure and molecular behaviour are being illuminated. For I think we may say that organic chemistry, moving closer to physics, is now passing from its classical period into a romantic one. It is becoming intellectually more adventurous in its concepts, more dynamic, and in its dealing with evidence more subtle. This it owes largely to the marvellously refined methods of modern physics as applied to the problems of molecular structure, and on the theoretical side to a further understanding of the nature of affinity and valency. The older organic chemistry obtained its clear picture

of the special relations among the atoms in a molecule, knowing but little of the forces which held them together, but the emergence into thought of the potentialities of the electron has, of course, entirely altered this position.

It is sure that organic chemistry with new equipment both in theory and practice has to-day, no less than other branches of chemistry, a great intellectual future before it. There is perhaps a warning desirable at the present moment, which cannot be better conveyed than in the words of Professor N. V. Sidgwick. "The chemist," he has written, "must resist the temptation to make his own physics; if he does it will be bad physics—just as the physicist has sometimes been tempted to make his own chemistry, and then it was bad chemistry." The methods and modes of thought of classical organic chemistry have this great advantage. The conceptions of molecular structure to which they have led, notwithstanding their limitations, are always to be tested by the supreme test of synthesis, and the greatness of organic chemistry as a science must always be based, in the main, on its ability to assemble atoms into any patterns it may desire. *Homo faber*—Man was born to construct.

I have reserved for my closing remarks certain considerations which on the present occasion I particularly desire to emphasise. I will venture first to speak for a moment as one concerned more particularly with biochemistry. Biochemistry has sometimes been called a hybrid science, but as hybrids are usually infertile I would ask you to think of it rather as a borderland pursuit. Being such, those who are devoted to it have to survey, so far as may be possible, the two fields of knowledge which meet at that borderland. It is difficult, however, save for a gifted few, to be as fully expert in either of those fields as those who work intensively in one alone. Experience shows, however, that those who explore scientific borderlands usually find work to do which would not be done by those whose main interests are confined to one of the fields in question. For historical reasons these fields have usually been longer cultivated than the region of the borderland. Departmentalism in science is almost inevitable and its disadvantages are familiar, but I want to emphasise the circumstance that where there is sympathy and trust between departments it may have advantages too.

Biochemistry has two basic needs. First, a knowledge as complete as possible of the physical properties and molecular structure of all the substances which play an essential part in the constitution of living systems. It could not be content with that knowledge alone if it is to justify its position as a separate branch of science. Its own special endeavour as a borderland science must be to study the chemical dynamics of living systems, to follow so far as may be possible the function and fate of each constituent amid the multitudinous reactions which underlie the manifestations of life, and the mechanisms which control these reactions. For this it is developing

its own to-day, and its present to-day, though difficult, is real. While it is true that in principle no student in land it is almost impossible for the specialized chemist to possess the knowledge and experience of the mode of the soft and firmness which made the accomplished organic chemist when determined to solve the structure of engaged in the difficult art of synthesis. If he has spent too much time in making himself a good biochemist in the line I have defined, he must usually remain at best an amateur in dealing with the problems of molecular structure. There never was a case in which the two can be better served by a more division of labour.

In the days of 1870, molecular organic chemistry and biochemistry took upon to reflect, but such as the tried progress of the art of synthesis in the history of the science that is understandable reason, the majority of organic chemists (though I and Fischer was an outstanding exception) seemed to take no special interest in natural products. From this circumstance the progress of biochemistry suffered.

But the last few years have witnessed a striking change in this attitude, for a number of our best and highest qualified organic chemists have devoted their energies to determine the intimate structure of natural products. I may mention, without exaggeration, the field of hormones and vitamins, substances of the utmost biological importance. The physiologists and biochemists discovered the existence of these and have dealt with their functions; it was for the most part in connection of their exact chemical nature. Now organic chemists with their special gifts have in a remarkably short time determined the nature of most of them, and some are already available on the market as synthetic products.

# THE CLINICIAN AND THE LABORATORY WORKER

(1931)

I greatly appreciate the honour involved in the invitation to address your important society, but I have some fear lest I may fail to justify the substitution of a laboratory worker for the clinician, who would normally be speaking to you on this occasion. The proper moment for an experimentalist to address a clinical audience is when he is possessed of a new message with practical bearings. I find, however, that most of the newer lines of investigation progressing just now in my department at Cambridge are either too academic or too incomplete to put before you. I propose, therefore, to deal with a subject which you may feel to be insurgent and perhaps repellent: the function of vitamins in human nutrition. But, although I am to say something about the practical importance of vitamin research, it will be chiefly to use the facts as a text for a wider discussion which I can only hope you will not feel to be too remote from your interests.

I would like to deal broadly with the relations between the clinician and the laboratory worker. To myself the attainment of a happy attitude of one to the other, involving mutual respect, has always seemed to be a matter of primary importance for the advance of medicine in this country, and I think I may assume that you are interested in the advance of medicine as well as in its practice. The importance of a good understanding may, to such as I, seem greater than it will to many of you. I have been in rather exceptionally close touch with the history of the relations in question and have always been interested in their psychological aspects. In the late eighties and the nineties of the last century I spent in one capacity or another no less than fifteen years in the medical school at Guy's Hospital. For part of that time I was in what was then an exceptional position in that hospital. I was in receipt of endowment for research while still engaged in the wards. In the earlier part of that period the divorce of the laboratory from the ward was almost complete, though, with the discovery of the tubercle and later of the diphtheria bacillus, the use of the laboratory for diagnostic purposes became a necessity and morbid histology called in a sense for laboratory work. But these activities were in the hands of junior clinicians, who thus filled their time of waiting for the more senior clinical posts. To any one, qualified or not, who became stamped as a laboratory worker the wards were closed and there was definite antagonism to his attempting to make any contact with cases. Succeding years slowly, but only slowly, altered this position.

When I went to Cambridge 33 years ago I lost all touch with clinical material, but I could not altogether lose my clinical interest or my concern about the influence of the laboratory.

Up to the time of the war one saw the influence of the laboratory gradually grow. It grew, indeed, faster during the war and for a few years after it. Then more recently came a certain reaction. Among thoughtful clinicians there arose a feeling, largely justifiable, that the increasing dependence upon laboratory reports for diagnostic purposes was spoiling the art and weakening the judgment of the clinician, and, still more justifiable, the realization that immature laboratory results were, when not misleading, much less valuable than the results of sound experience and direct observation at the bedside. Among those who voiced this feeling was the late Sir James MacLennan, whose own brilliant bedside work gave him authority to speak. It has lately been voiced, but in less justifiable terms, by Lord Moynihan. To be frank, I think it is a certain irritation induced by Lord Moynihan's recent utterances, probably familiar to you, which has led me to address you on the lines I have chosen. His complaint that laboratory activities have become too remote from actuality is not justified. He forgets that in the progress of science the emergence of some one fact which has immediate practical application may inevitably need the previous acquisition of knowledge which itself may have no immediate practical value. In solving a practical problem, let us say the control of cancer, the frontal attack is seldom successful. It is by flank attacks and gradual undermining that the position is captured. That there should be more clinical research than at present is a desire that is justifiable; but when we come to inquire what precisely is meant by clinical research we find that in so far as it is really research the distinction between it and laboratory research tends to disappear.

Apart from such views as those expressed by Lord Moynihan, one cannot deny that there is a certain mild antagonism, not usually outspoken or venomous, between the average clinician and the average laboratory worker concerned with the medical aspect of science. I have many friends in both categories and I cannot but be aware that there is some misunderstanding. The former resent a certain intellectual conceit on the part of the latter, and realize that in his ignorance he ignores the most important side of practice, the art as distinct from the science of medicine. The laboratory man, on the other hand, is apt to feel that the clinician is often unprogressive and too content with empiricism, and, to be quite frank, he resents a certain patronage based upon a sense of greater social importance on the part of the doctor. This last is a small matter though it is not unreal; but I should not refer to it here did I not wish in my closing remarks to deal on much broader grounds with the position of the professed investigator in this country. In respect to the misunderstanding that I suggest exists I would only add this: the physician may sometimes unjustly undervalue the services of the laboratory, but the scientist who pretends to despise the work of the physician is merely foolish. He forgets that the practice of medicine calls for personal qualifications that need not be his own. In that practice knowledge has to be applied amid the intricacies of human

nature, and must ever take account of the prejudices, the sensibilities, the hopes, fears, and reserves of complex human beings. Moreover, the physician has to deal with the reactions of the human body as a whole, a very different thing from dealing with simplified and individualised phenomena such as the experimentalist secures for his own convenience.

Yet, as you will readily understand, my intention is nevertheless to speak in praise of the laboratory. For the moment I would ask you to distinguish between the laboratory as an aid to diagnosis and its functions as the actual progress of medical knowledge. It is the latter which is my main concern.

I must here refer to the title of my address. Knowledge of Nature is gained both by observation and experiment, but progress by means of the former, and much older, method is almost always slow, while by the latter, and much newer, method it may sometimes be very fast. The essence of an experiment, of course, is that you narrow an inquiry to a single issue, that in studying phenomena you vary only one contributory factor at a time. You study the phenomenon in the presence of that factor and in its absence; and you get an unequivocal answer as to the influence and importance of that factor. When an observation has in this sense a "control" it becomes an experiment.

Forgive me if I am putting the obvious before you; it is a point which sometimes needs emphasis. I would emphasise it by referring to a chapter in the history of medicine. In the middle of the last century this country possessed physicians who were exceptionally fine observers. Among them was William Jenner. Now Jenner had been striving to make it clear to his English colleagues that typhus and typhoid fevers were distinct pathological entities. His views, though of course sound and correct, met with opposition and criticism. "I considered, therefore," he writes in the preface to his book on fevers published in 1850, "that it was necessary to begin *de novo* and consult only the voice of nature—convinced that although the most intellectual might fail at first to comprehend her often ambiguous language, yet that her most humble votaries might by patience and daily watching, by keeping honest record of every sound she uttered—by joining letter to letter, adding word to word, and line to line—at last spell out her meaning and so reach that rank which the great master of induction tells us that man may legitimately hope to attain, namely that of her interpreter." Here in fine terms we have an epitome of what the method of the observer should be. If progress with such high ideals is not rapid it must be due to limitations in the method. It cost Jenner many years of work to convince his contemporaries by the evidence he produced. Jenner felt the ambiguity of nature's chance remarks but saw apparently no remedy save in the laborious recording of her spontaneous utterings and a painful effort at interpretation. Nature, however, can be taken into the witness box and directly questioned; moreover, the questions can be

so put that there is no possibility of ambiguity in the answers. This is the method of experiment. It was the method of Pasteur.

Don't, I beg, suppose that I undervalue the work of the observer, and especially the observer who has the leisure and energy to record his observations. Without such record his experience dies with him and he makes no permanent contribution to knowledge. But recorded observations at the bedside have, of course, high value, especially such careful classification of records as are characteristic of the work of men like Sir James Mackenzie. The careful description of individual cases and the statistical treatment of such records are essential to progress. Moreover, every experimental result from the laboratory, which claims to affect medical practice, can only be accepted as significant when its reality has been tested by the clinician at the bedside. It yet remains true that only the method of experiment can yield rapid progress in fundamental knowledge.

Such knowledge as the first recognition of the importance of micro-organisms, the first steps in immunology, the existence and functions of internal secretions, and the like, could not have arisen from observations on the intact body. Forty generations of observers failed to arrive at that sort of knowledge; some three generations of experimentalists produced it. In appraising the present fruits of experiment I would, indeed, have you remember how short in comparison with the sum total of human experience has been the period of its application. It has scarcely begun to exert its influence.

Keeping to the same illustration, think of the development of knowledge concerning fevers during the century which preceded the experiments of Pasteur. The bedside studies of such men as Louis, Bartlett, Murchison, and many others made for sound views and for a healthy recognition of ignorance. Descriptions became more accurate; the significance of this or that symptom became better evaluated as the result of statistical records, and a sharper classification became possible. Nothing was added, however, which appreciably widened the point of view; nothing that could remove the utter vagueness concerning the essential nature and cause of fevers, or throw light on the mysteries of contagion. In this respect the knowledge of the middle of the nineteenth century scarcely differed from that of the days of Sydenham. To a new era belonged that moment when Pasteur, intruding into a vague discussion concerning the "cause" of puerperal fever, rushed to the blackboard, made a rapid sketch of a microbe and cried "Tenez, voici sa figure!" It is a great thing to see the face of the enemy.

As I warned you I have chosen the nutritional or dietetic field in which to illustrate the importance of experiment. I don't know if I am right, but I cannot help feeling that no demand on the part of a patient for advice involves more embarrassment for a scientifically-minded physician than when it concerns questions of diet. Advice on this matter must usually be highly empirical. Let me be bold enough to suggest that when given it is often based upon views and preconceptions which lack any foundation

of evidence. I am not thinking of the needs of dyspeptics; dietary adjustments in such cases form a matter for observation on the part of the doctor and patient combined. I am thinking rather of the question as to how far the nature and quality of food may affect health fundamentally. I am personally convinced that they can, and I want to remind you of the evidence which seems to justify such belief. I must perhaps admit that I am thinking more of the future than of the present; for the body of knowledge in question is far from complete, though it comprises many well proved facts and teachings of immediate importance.

The rest of this address is lacking.—Eds.

## THE PROBLEMS OF SPECIFICITY IN BIOCHEMICAL CATALYSIS

[33rd Robert Boyle Lecture, 1931]

To be allowed to deliver a Boyle Lecture under the auspices of your famous Club\* is a privilege which every devotee of science may well covet. While Oxford is especially entitled to cherish the memory of Robert Boyle, his outstanding personality can never be forgotten by any of those who are familiar with the history of science. I am unacquainted with any portraits of him that Oxford may possess, but, as many here will know, there is in the rooms of the Royal Society a very fine one by Kerseboom. I have stood so often before that picture that I seem to have grown familiar with the personality it so vividly portrays. One finds there, I think, not only the high intellectual qualities which one would expect, but also those finer aspects of character which were characteristic of the man portrayed.

Boyle took, as you will know, an abiding interest in the work of the Royal Society, which he helped to found, and in its archives there are many records which show the breadth of his scientific interests and the generosity which was part of his nature. The circumstances in which he refused the Presidency illustrate the character of his mind. He was duly elected to that office in 1680, but because the Society's Charter demanded that the President should take the Oath of Allegiance and the Oath of Supremacy, a necessity which was not in accord with his conscience, he refused the Chair. He communicated this decision in a letter to Robert Hooke, then one of the Secretaries. He there says of his reasons: ". . . they are of such weight with me who have a great (and perhaps peculiar) tenderness in point of oaths that I must humbly beg the Royal Society to proceed to a new election." The Chair then went to Sir Christopher Wren. The letter in question gives evidence not only of Boyle's high standard of conduct, but also of his essential modesty. Boyle, indeed, lacked none of the qualities which make for true greatness.

You will be aware that his ostensible intention in writing the *Sceptical Chemist* was to convert his contemporaries from the hide-bound view that the material world, organic and inorganic, is constructed from the three so-called hypostatic elements: salt, sulphur and mercury. The book in reality called for a more general reform in chemical thought. It urged chemists to take more note of the dynamic side of phenomena.

In his introduction Boyle has a passage which may serve as an introduction to what I have to say this evening. "There are," he says, "a multitude of accidents relating to the human body, which will scarcely be clearly and

\* The Oxford University Scientific Club.

satisfactorily made out by them that confine themselves to deduce things from salt, sulphur and mercury, and the other notions peculiar to the chemists, without taking much more notice than they are wont to of the motions and figures of the small parts of matter and the other more catholic and fruitful affections of bodies." Chemists and physicists for a long time past have been taking full notice of the "motions and figures of the small parts of matter," and Boyle would have fully approved of the spirit of pure chemistry of to-day. I would have you observe, however, that in the passage quoted he was speaking of the chemistry of the human body; his thought at the moment was biological. Now, I find even to-day a tendency among some at least of my chemical colleagues to view the aims of biochemistry as though they were to be satisfied by the mere isolation of biological products and the determination of their constitution; to be content, that is, with "figures" and ignore "motions." If, as I feel, the pure chemist sometimes seems to lose sympathy with biochemistry when it turns from constitutional problems to the dynamic events of metabolism, it may be due in part to relative unfamiliarity with the nature of the problems presented by the latter, and perhaps also to some mistrust of the methods applied to their solution. These methods are in certain respects different, and at present often inferior, to his own. Biochemistry is, however, providing itself with methods which are adequate for its immediate problems and is almost daily improving them.

While constitutional studies may be, and, till lately, usually were, an end in themselves for classical organic chemistry, they are, clearly, for biochemistry, rather a means to an end. If the latter is to justify itself as a self-standing branch of scientific inquiry its task is to study dynamic events as they occur in living tissues. The knowledge of constitution provided by the methods of organic chemistry is, of course, a prime essential for such studies, and the data concerning colloidal systems provided by modern physical chemistry are a necessary adjunct; but a colloidal system in which no active chemical changes are present, comprising energy-yielding reactions, is remote from a living system. Colloidal systems would seem, however, to be the only form of apparatus in which the multifarious reactions which organic molecules undergo in the living organism could be so remarkably organised and co-ordinated. It is the nature and course of these reactions and the factors which control them that the biochemist must endeavour to study. That catalysis, in the broadest sense of the term, determines each individual reaction would now seem sure, and, since the living cell is a heterogeneous colloidal system, surface catalysis may be expected to dominate the field of activities.

It was frequently urged in the past that the dynamics of living matter must always remain beyond the reach of chemical studies, since at the moment when chemical methods are applied to them the materials cease to be alive. That the impasse thus suggested, with apparent logic, can be eluded has been shown by the substantial progress of biochemistry

during the last thirty years. True, the biological chemist, like other experimentalists, must simplify his experimental conditions so far as legitimately he may; he must deal piecemeal with isolated parts of the complex systems which it is his ultimate task to describe. Doubtless, moreover, in an endeavour to deduce the nature of the whole from his studies of parts he must be content, for a very long time to come at least, with a synthesis which can only be intellectual. For a material synthesis would have to be not the chemical synthesis of a single substance, however complex, with the properties of life, but the construction of a highly elaborate system. I would like to express my belief, however, that progress, even on the lines of present efforts with legitimate inferences from the results, will reach to a conception of the living cell as a physico-chemical system which, if incomplete, will be free from elements of falsity.

One line of experiment among many others that the biological chemist has open to him, and one that has been much employed, is to make whenever possible preparations from a tissue, or from free living cells, such that a single chemical event (one reaction or a few related reactions) characteristic of the living tissue or cell, can be isolated from others and studied *in vitro* in respect of the material changes involved, and when possible in respect of kinetics. Such a preparation will in general necessarily contain not alone the substance which undergoes change (this may be already present, or added in known amounts), but a suitable catalytic agent extracted from the cells; that is, a substrate and an enzyme.

Now whenever such isolated systems are studied it is commonly found that enzyme and substrate are in a very close adjustment; the activity of the former if not confined to the single substrate has a very small range of specificity. Recurring to Boyle's quaint phrases quoted earlier we may say that in the case of enzymes and substrates—"affections" are "fruitful" but not "catholic"! If this remains true of events in the intact cell, then, since the reactions which proceed in a single cell are exceedingly numerous and certainly cannot proceed unless the molecules are in some sense activated, it follows that a very minute chemical system must contain an extraordinarily large variety of catalytic agencies. The acceptance of this conclusion represents to many an intellectual difficulty. This difficulty is the text of my remarks. It has been often discussed, but I would ask you to consider it in the light of fresh facts.

It may be well just to remind you, at this point, of the orders of magnitude which living cells display. I will take a few instances at random.

Hepatic cells: diameter  $20\ \mu$ ; weight  $10^{-7}$  gm.

Human red blood corpuscles: diameter  $7\ \mu$ ; weight  $10^{-10}$  gm.

Yeast cell: diameter  $2.5\text{--}10\ \mu$ .

Average bacillus: base of cylinder  $1\ \mu$ , height  $2\ \mu$ ; weight  $10^{-12}$  gm.

The solid matter in 500 million individuals of *Bacillus coli* weighs 0.1 mgm.

I may next remind you how diverse are the changes which organic molecules may undergo within chemical systems of such small magnitude. Prominent in the cell, of course, are the reversible processes of hydrolysis and condensation, and irreversible oxidation-reduction processes; the former as the basis of events largely unrelated to the liberation of energy; the latter yielding the energy for physiological work. Both types of change are suffered by a variety of substances. In addition to these major reactions, though commonly with their intervention, organic molecules may suffer alkylation and acylation, amination and deamination, and may enter into a variety of syntheses. Although a more specialised event even halogenisation may occur, as in the synthesis of thyroxine in the thyroid gland. In brief, all those varieties of treatment, with the fewest of exceptions, which substances suffer in the organic chemist's laboratory, they may suffer in biological systems, and that, of course, at a temperature of 37° C. and in media which are nearly neutral. If the progress of each reaction depends upon the influence of a specific catalyst the variety of these must indeed be great. It is desirable perhaps that I should deal with a few illustrative cases of such specific relations.

I will speak of the catalysts we are chiefly to consider as enzymes, since the use of the term is so usual, though it must be used in a sense perhaps unduly wide if it is to cover them all. It is well to remember that such agencies as hormones and vitamins may exert catalytic functions of unknown nature. These, however, we are not to consider.

The study of hydrolytic enzyme-systems, though of course not new, has been greatly intensified of late under the influence of the work of Willstätter and his school. In the domain of carbohydrate hydrolysis there are, because of the abundant possibilities for isomerism, great opportunities for the illustration of specificity. It was Emil Fischer's classical work upon the  $\alpha$  and  $\beta$  glucosides, and the nice adjustment of enzymes to each of these types respectively, which first awakened general interest in the specificity of these agencies, and led to his use of the "lock and key" analogy. Since then a great number of enzymes hydrolysing carbohydrates have been studied (my colleague, Professor J. B. S. Haldane, has listed some thirty in a recent monograph), and, though the range of activity discovered varies from case to case, the phenomenon of high specificity is abundantly illustrated. Of course not all, or nearly all, of these enzymes are contained in, or produced by, any individual cell, but any cell may contain several. Of special interest as illustrating my subject is the recent proof that in the complete hydrolysis of complex protein molecules a considerably greater variety of enzymes is concerned than till now was suspected. As breakdown proceeds new molecular species appear in solution, and, owing to its specific limitations, an enzyme active at an earlier stage may be out of harmony at a later stage, to which, however, one of its congeners then proves itself to be adjusted. Although such facts have emerged chiefly from a study of digestive processes, and refer, therefore, to enzymes which act outside

cells, yet we must remember they are produced in and may remain in cells. Indeed, we have much more than suggestions for the belief that within every cell protein metabolism is controlled by a variety of enzymes. The remarkable circumstance that every tissue unit during growth or repair builds up from a medley of amino-acids its own peculiar proteins, may depend upon the control of successive synthetic stages by such specific catalysts.

I do not, however, propose to refer further to hydrolytic enzymes, but will more closely illustrate my theme by reference to the catalysts which promote oxidation and reduction in cells and tissues. Knowledge concerning these, of an informative kind, has been won but recently. Of these one type is of special interest. When a preparation of any one of the enzymes of the type to which I allude is added to an aqueous solution of its appropriate substrate, duly adjusted with respect to hydrogen ion concentration, oxidation of the substrate proceeds actively in the presence of oxygen, though the substrate may be a substance highly resistant to oxidation by laboratory methods. An enzyme-substrate system of the kind in question is characterised, however, by the circumstance that the oxidation proceeds equally well, and on similar lines, when the place of oxygen is taken by any one of a number of reducible substances. In each case the general result is that pairs of hydrogen atoms are transferred from the substrate either to oxygen or to the alternative oxidising (reducible) substance. Convenience has led us to speak of the latter as hydrogen acceptors and of the substrate in each such case as a hydrogen donator.\* The above facts show that this type of catalytic agent is not concerned with any form of oxygen activation, but rather with making the substrates susceptible to oxidation. Such catalysts have become known as "dehydrases" or "dehydrogenases"; the latter being perhaps the better term. We owe to the School of Thunberg in Lund our first realisation of the general importance of dehydrogenases in the biological field.

The phenomena observed in the systems in question are in full accord with the views of Wieland concerning the oxidation of organic molecules in general, in that we may here, if we wish, speak of the "activation" of hydrogen as contrasted with the "activation" of oxygen. In Wieland's view, as at first put forward, such activated hydrogen is assumed to be in all cases capable of direct union with molecular oxygen. Studies of biological material during the last ten years make it necessary, however, to avoid thinking in terms of this clear-cut antithesis between "hydrogen activation" and "oxygen activation" as the essential determinant of oxidation. When a sensitive molecule is in contact with an appropriate dehydrogenase its affinity fields are so altered that certain hydrogen atoms are in some sense mobilised. In particular cases these are then able, as we are to see, to unite directly with molecular oxygen. In other cases, however, a dehydrogenase-substrate system, though it may freely reduce such a substance as, say,

\* Some will prefer to think in terms of electron and proton transference. It is doubtful, however, if at the present moment discussion becomes clearer by this change of language.

methylene blue, and must, therefore, contain active hydrogen in Wieland's sense, is incapable of reducing molecular oxygen. A further agent, an oxygen activator, or a hydrogen "carrier," must then be present to complete an active system.

In order to illustrate the statements I have just made, two enzymes of the dehydrogenase type may receive some further reference. One of these determines the oxidation of succinic acid to fumaric acid, and the other the oxidation of xanthin and hypoxanthin to uric acid. Very active preparations of either can be made, and it may be noted that both oxidise molecules which are particularly resistant to oxidation by laboratory reagents. Each is highly specific in its relations with its substrate. In each case oxidation proceeds with readiness when oxygen is replaced by a reducible substance such as methylene blue. Differences, however, are displayed by preparations containing these two catalysts, which are significant in that they illustrate respectively the properties of two types of dehydrogenase, or perhaps rather a difference in the contents of the various preparations we make of these enzymes. I must refer only to the most important of these differences as an example of a distinction already noted. When the purin-dehydrogenase is in contact with its substrates we have good evidence to show that the system transfers hydrogen direct to molecular oxygen. Preparations containing the succinic acid dehydrogenase may appear to show a similar property, but this is because the enzyme is then still associated with other factors, to be mentioned immediately, which promote the union with oxygen. When the activity of these latter agencies is inhibited—as by the addition of cyanides—the system reduces, say, methylene blue, and therefore contains active hydrogen, but it does not react with molecular oxygen. The reason for this essential difference between two types of dehydrogenase is not wholly clear; those which need the assistance of an oxygen-activator are much the commoner.

The purin enzyme (or "xanthin oxidase") may receive further brief reference as its properties are usefully illustrative. Its associations in the cell are a reminder of the specificity and multiplicity that we are considering; for in the breakdown of nucleic acid in the animal body, after specific hydrolytic enzymes have liberated the purin bases guanine and adenine, a separate enzyme functions in the deamination of each of these and then the enzyme under reference next determines the oxidation of the resulting xanthine and hypoxanthine to uric acid. In most mammals uric acid is further oxidised to allantoin by yet another specific agent, and finally an oxidative enzyme has been described which opens the ring of allantoin. The xanthine oxidase shows high specificity within the purin group. Only two other purins, closely related to xanthin, namely, 6,8-dioxypurine and thioxanthine are oxidised under its influence. Insertion of a methyl or amino group in any position completely prevents activation. This enzyme is one that in physiological circumstances leaves the cells from which it takes origin and in which it functions, for it is found in relatively high

concentration in milk. From this fluid very active aqueous solutions can be prepared in which it shows high colloidal dispersion. M. Dixon has studied the kinetics of the systems it forms with its substrates. In this study several interesting points were revealed. I will, however, only mention the circumstance that the system is one of those in which increase in the concentration of the substrate beyond an optimum slows the velocity of the reaction. We can most easily picture as the reason for this the circumstance that the enzyme surface becomes so completely occupied by the substrate molecules that there is no room for oxygen molecules to assemble at that surface, a point of interest to theories of enzyme action. The phenomenon is met with outside the biological field. I have mentioned these details here, however, chiefly to convince you that in these dehydrogenase catalysts we have to do with biological agencies susceptible to objective study, and that they display the specificity with which I am dealing. Several dehydrogenases with specific activities have been identified, and it is sure that every typical living cell contains many. I may mention, for instance, that J. H. Quastel, lately my colleague, when studying such phenomena in the case of bacteria found that under the influence of *Bacillus coli communis* no less than 56 substances were activated out of 103 investigated—activated in the sense that they became either hydrogen donators to various hydrogen acceptors or, conversely, became themselves acceptors of hydrogen; that is to say, oxidising agents. All are wholly inert in either sense in the absence of the organism. It is noteworthy that all these activations appear to take place at the outer surface of this very small organism. The question of specificity here will receive mention a little later.

Paradoxically, it may best help to a realisation of the significance of specificity in biological catalysis if one considers the nature of the functions of those chemical mechanisms in the cell which are not specific, or are much less specific, in the sense under discussion. I have already pointed out that most dehydrogenase systems do not transfer hydrogen direct to molecular oxygen. The latter must first be activated or at any rate brought by some mechanism into the field of activity. Recent research, and especially that of Keilin at Cambridge and of Warburg at Berlin, has thrown much interesting light on these mechanisms.

Metallic iron has long been suspected of playing a prominent part in the promotion of biological oxidations. The work of the authors mentioned has shown that this is only (or especially) true of iron when in association with a tetrapyrrol nucleus; metal compounds of this type proving to be of prime biological importance. We have long known that a tetrapyrrol grouping is shared by blood pigments and chlorophyll, and we now know that it forms the basal structure of agencies which promote oxidation within the living tissues, and also of the agent—catalase—which exercises the secondary function of controlling the concentration of hydrogen peroxide which may appear during such oxidations. The properties of this particular molecular grouping play a fundamental part, therefore, first in the securance

of solar energy for the whole field of biological activities; secondly, in the transport of molecular, inactive, oxygen from the lungs of higher animals to their tissues; and finally, in "activating" this molecular oxygen within the tissues themselves. Such diverse activities involve, of course, minor modifications in the fundamental structure. We have here a circumstance which is, I think, characteristic of the biochemical field. Amid the complex activities of the cell we find a certain chemical economy. Modifications, sometimes relatively slight, in a fundamental molecular structure may yield diverse substances which as constituents of the cell have very various parts to play. This should be in our minds when we are thinking of the multiplicity of enzymes. Of the structure of the tetrapyrrol nucleus we have now, owing more particularly to the labours of Kuster and Hans Fischer, highly adequate information.

In discussing the influence of these iron-pyrrol compounds in promoting oxidations I will deal first with the *cytochrome system* of Keilin. In spite of its great importance I must treat it very briefly. Suffice it to say that in this system we have a group of three substances, widely distributed in living cells and tissues, which are beyond question iron-pyrrol derivatives (atypical "haemochromogens"). For brevity's sake, and because one of them is the most important, I may here treat them as one and speak of "cytochrome." Because of associated changes in its spectrum, the oxidation and reduction of cytochrome may be easily observed in intact cells. It is reduced with great readiness by reducing systems in the cell, and it is likewise freely oxidised in the cell, though only under the influence of another agency. This latter has long been known as an oxidising agent of the tissues, though before Keilin's studies its true significance was not recognised. Because of a colour reaction which indicates its presence it has been known as the indophenol oxidase.

It is important to realise at this stage that the specific dehydrogenase systems are the most important factors concerned in the reduction of cytochrome. Thus cytochrome, activated by the indophenol oxidase, acts as an intermediate carrier of hydrogen to oxygen from the various substrates activated by dehydrogenases. To use a term employed by Sir Charles Sherrington with reference to the course of nerve impulses, the system constitutes "a final common path" for hydrogen transport.

I will next deal, though again very briefly, with the brilliant researches of Otto Warburg, who is the leader of a school which has looked to the activation of molecular oxygen as the one essential determinant of biological oxidation. Warburg holds, indeed, that one individual agency controls the whole field of oxidations. This, the "respiratory enzyme (*Atmungsferment*)," is again a substance in which iron is linked to the nitrogen of a pyrrol derivative.

I must not stop to put before you the logical sequence of experiments and deductions which have led Warburg to this view, but only touch upon the latest, and most conclusive, evidence.

It has long been known that carbon monoxide is an inhibitor of respiration in the higher animals. In relatively low concentrations it is capable of displacing oxygen from its union with the iron of haemoglobin, thus interfering with oxygen transport in the blood; but earlier observations seemed to show that it does not inhibit oxidative events in the tissues themselves. Warburg has shown, however, that in high concentrations (at pressures, for instance, near to one atmosphere), it may inhibit cell respiration as a whole. But as in the case of haemoglobin its effect here depends also on the concomitant oxygen concentration. The lower the oxygen pressure the more pronounced the inhibition of respiration at a given carbon monoxide pressure.

You will perhaps here recall that J. S. Haldane several years ago showed that CO-haemoglobin is decomposed into CO and haemoglobin by light, and Warburg's colleagues have shown that the CO-compounds of certain simple iron-pyrrol derivatives are decomposed in the same way.

Now Warburg, dealing first with yeast cells, but afterwards extending the observation to a variety of cells and tissues, proved that, when the respiration of these (and respiration summarises the effective oxidations which occur in a tissue) has been inhibited by CO, illumination of the living materials restores it. The phenomenon was studied quantitatively, and it became clear that some compound in the cell, controlling respiration, enters into association with oxygen and carbon monoxide in a proportion depending upon their relative pressures, and that light decomposes the CO-compound, a circumstance so highly characteristic of iron-pyrrol derivatives. It is the ratio  $\text{CO}/\text{O}_2$  which determines the degree of respiratory inhibition. A constant ( $K$ ) was determined expressing the relative affinity of the responsible catalyst for CO and  $\text{O}_2$  respectively:

$$K = \frac{n}{1-n} \cdot \text{CO}/\text{O}_2,$$

where  $n$  is the ratio of respiration in the presence of CO to the respiration in its absence. The value of  $K$  for yeast in the dark was of the order of 10, while in the light it was above 100. This cell constituent then is Warburg's "respiratory enzyme," and he has made most ingenious use of the relations described in an endeavour to decide upon its nature. It is present in very low concentration and shows no visible spectrum. Warburg, however, determined the relative effectiveness of light of different wavelengths in restoring cell respiration after its inhibition by CO, and obtained a curve showing how this effectiveness is related to wavelength. Increased respiration measures increased dissociation. On the assumption, justified thermodynamically, that light must be absorbed by the carbon monoxide compound of the enzyme in proportion to its effectiveness in dissociating it, Warburg was able to claim that the curve in question must represent the actual absorption spectrum of the enzyme. Further, since the time required to split off the CO must be related to the absolute light absorption by the

compound, he was able, by introducing time measurements into his experiments, to determine the absolute absorption coefficient for any particular wavelength.

Now the absorption curve actually obtained on these lines closely resembled that of certain iron-tetrapyrrol compounds. In one of his latest publications Warburg has shown, indeed, that the CO compound of Willstätter's pheophorbide (a derivative of chlorophyll in which Fe has replaced Mg) gives a spectrum which resembles that of the enzyme very closely indeed. Further experiments on similar lines may well lead to the discovery of a compound giving an identical spectrum. If so, Warburg will have determined the chemical nature of a biological catalyst without having removed it from the living material!

There exists biologically at least one other final path besides that of the cytochrome-oxidase system for the transport of hydrogen to molecular oxygen. Certain tissue agencies can reduce cystine to cysteine; the disulphide group of the former to the thiol group of the latter. On the other hand, in the presence of traces of iron, cysteine is reoxidised to cystine by molecular oxygen. I have myself shown that animal tissues, yeast, and other cells contain a tripeptide of which cysteine is a constituent amino-acid. When this tripeptide is isolated in crystalline form the thiol group of its constituent cysteine is oxidised by molecular oxygen only if traces of an organic metallic compound are present, and this apparently is neither the cytochrome system nor Warburg's enzyme. When in the living cell, with its normal associations, the thiol group of the substance (which I have called glutathione) is, however, freely oxidised by molecular oxygen, and during the processes of respiration this oxidation is balanced by the continuance of reduction processes, so that transport of hydrogen to oxygen must proceed by this path. Quantitatively it is much less prominent than the cytochrome path, but apparently it is essential to the normality of the cell. I mention it because, so far as yet ascertained, the dehydrogenases which reduce cytochrome do not reduce the sulphur group of glutathione—other agencies are concerned. This circumstance may be a question of reduction potentials, but in any case it indicates a certain specificity among relations in the cell other than those of enzyme and substrate.

I remarked just now that the functions of less specific catalysts in the cell may illustrate the significance of the functions of highly specific catalysts. Warburg, whose brilliant experimental work entitles his opinions to respect, takes, as I have said, the view that the activation of molecular oxygen is the one essential factor in the induction and control of biological oxidation. His iron-pyrrol catalyst controls the processes of respiration in living tissues, and if I may summarise his teaching, briefly and crudely, it is that in the study of respiration all else is incidental and unimportant. But is it not clear that the mere arrival in the field of active oxygen, capable of dealing with all available oxidisable substances in the cell, could not result in the highly organised processes which are so characteristic of life? It is

noteworthy in the first place that many substances prone to easy oxidation in the laboratory are stable in living tissue, while others highly resistant to oxidation are quickly oxidised. In the fact that the substrates to be oxidised must first suffer activation, and that by specific agents, we have one reason at least why orderly and organised events occur. The concentration of individual catalysts is duly adjusted, and therefore the diverse corresponding oxidations proceed with adjusted velocity. There can be little doubt that specificity in catalysis plays a real part in determining the high organisation of chemical events in the cell. It is certain at least that activation of the molecules to be oxidised and the activation of oxygen are alike essential for the progress of most biological oxidations.

Here, however, I would again raise the question as to how far the extreme complexity of catalytic mechanisms in the cell, which all that I have said seems to involve, should compel intellectual disbelief. Are the conclusions, for some reason, erroneous?

If, when thinking of Quastel's observations, to which I earlier referred, showing that the minute outer surface of a bacillus can activate at least fifty distinct substances, we have to believe in a specific area concerned with each activation, we certainly must picture that surface as a very remarkable mosaic. The existence of specificity is clear enough, for by suitable treatment of the cell surface we can destroy its power for certain activations and leave others intact. Moreover, at least one dehydrogenase has been separated from such a bacterial surface (by Miss M. Stephenson), that is activating lactic acid; and this when separated proved to be specific enough. Nevertheless, there is some evidence which I must not stop to put before you, that in the intact living cell each catalytic unit of surface may have a somewhat wider range of activity than at first suggested.

Quastel himself, indeed, has brought forward a theory of activation at cell surfaces which might seem to mitigate some of the demands for extreme specificity. Associated with certain groups in the cell membrane there exist (on this theory) electric fields of varying strength due to unsatisfied affinities in localised regions of the surface. If a molecule enters one of these fields the field will tend to divert the electrons and protons from their normal orbits, and this, should the molecule possess certain special elements of structure, will result in activation. With a given strength of field, one molecule will be activated and another not, and apparent specificity of surface might then be largely interpreted in terms of the strength of field and the properties of the molecule to be activated. Quastel finds, however, that for the latter to remain in the field for a period adequate for activation it must be specifically adsorbed in the neighbourhood of the field, and it would seem that only the locus of specificity and not its actuality is removed on such a view. The theory calls, nevertheless, for full consideration.

Experimental studies make it clear enough that the different catalysts of the cell offer varying degrees of resistance to extraction from the system. We speak of soluble and insoluble enzymes. The latter are attached to,

or form part of, actual histological structures, the former are probably part of the fluid cytoplasm and are more susceptible of study as individuals. I would like to suggest that if we think of the soluble enzymes in particular it may be felt not too difficult to form a picture of how a minute chemical system like the living cell may contain a great number of catalysts. Indeed, the very smallness of the cell is a help to form such a picture. Notably, as A. V. Hill has pointed out, the factor of diffusion which, as a slow process, may prominently intervene in controlling the velocity of change in macro systems becomes, in the case of the cell, altogether negligible. Another point to bear in mind here is the high water content of the cell. In the case of typical cells such, for example, as the ovum, or the less differentiated cells of animal and plant tissues, we find that the greater part of them consists always of the optically homogeneous cytoplasm, or hyaloplasm. This is associated, of course, with one fundamentally important set of differentiated structures, the nucleus and its appendages. Also differentiated from the cytoplasm are commonly other structures, but these are less fundamental as they are not necessarily found in every cell. The influence of the nucleus in determining the biological properties and behaviour of a cell is fundamental, and probably its constituents may exert special catalytic functions, though on this point we have little knowledge.

Considering, however, the quantitatively prominent cytoplasm, few will now deny that its more superficial physical properties are those of a "lyophil" colloidal system. Whatever else it may be, it is a physical system of that kind. Whereas, however, in the non-living systems of the same kind which are studied by the ordinary methods of physical chemistry the surfaces which separate the internal from the external phase are generally uniform in kind and exert no more than ordinary surface effects upon dissolved molecules (if any such be present in the system), the micellae or particles which form the internal phase in the cytoplasm *must* be diverse in respect of their surfaces, and a proportion, probably no small proportion, of these surfaces, must have catalytic properties. I say "must" because we can so often obtain simple aqueous extracts of cells or fluids expressed from them, consisting essentially of cytoplasmic constituents, and find that a number of the normal chemical reactions, of course with diminished velocity, still proceed therein.

Now remembering that the cytoplasm of the cell contains 75 per cent. water, and that much of this is "free," we must believe that many substrate molecules in the fluid phases are able to display their kinetic properties; contacts between them and the dispersed particles must therefore continuously occur. Only, however, when a substrate meets with a particle possessing a catalytic surface adjusted to its own structure will it be activated. The frequency of such contacts will, of course, depend upon the concentration of the substrate and upon the number of the dispersed particles possessing that particular surface. This is in one sense to say no more than that the velocity of any particular reaction depends alike on the concentration of a substrate and on that of the adjusted enzyme. What I want to urge, however, is

that, although the microscope may detect no heterogeneity in the cytoplasm, the colloid particles dispersed in it must have diverse surfaces and may, even in relatively large proportion, be essentially diverse enzymes.

My colleague, Professor J. B. S. Haldane, has calculated that in a yeast cell the numbers of saccharase molecules (or particles carrying the saccharase structure) is probably less than 150,000, but probably not less than 15,000. In some other cells he suggests they may not be more than 1,000. Data are, indeed, accumulating which will justify the application of mathematics for testing assumptions such as those I have just made. Haldane, indeed, in an unpublished paper has made a preliminary investigation of the kind. I may mention two of his rather startling results. One is that work on enzymes has suggested that collision frequencies in a liquid may be made greater than would be the case if the gas laws held good. Another is that the average atom on its metabolic path within a living cell may be dealt with by more than 100 catalysts in succession.

Some of my audience may feel acutely the intellectual difficulty which it has been the purpose of this lecture to discuss; the difficulty, namely, that is involved in the belief that a minute chemical system like the living cell can comprise within itself so great a multitude of distinct catalytic agencies. For such I cannot be sure that considerations of the above very superficial kind will be of any assistance. I must confess, indeed, that I did not set out to decide in this address how far the difficulty in question is real; but rather to state the problem afresh and remind you that it calls for thought at the present time, when the number of individual enzymes isolated is steadily increasing.

It should be remembered at any rate that only thirty years ago the conception of intracellular enzymes as general determinants of reactions in living tissues was new. The much older knowledge of extracellular enzymes in the digestive juices did not for many years suggest these much wider functions for such catalysts. Explanations of cell dynamics in so far as they were attempted were of an obscurantist type, giving little or no lead for experiment. The recognition that these catalysts function in living processes represents progress that is very real, but progress, as usual, has revealed new problems.

The task of the biochemist wishing to get to the heart of his problem is exceptional in that he must study systems in which the organisation of chemical events counts for more, and is carried far beyond, such simpler co-ordinations as may be found in non-living systems. He would be over-bold were he to claim at present that such high organisation can depend alone upon adjusted concentrations and ordered structural distribution among specialised colloidal catalysts, but he is justified, I think, in feeling sure that such factors contribute to that organisation in a significant sense. The biochemist, when he aims at describing living systems in his own language, comes in contact with philosophical thought. Current philosophy is busy in emphasising the truism that the properties of the whole do not

merely summarise but emerge from the properties of its parts, and some exponents hold *a priori* that biochemical data can throw no real light on the nature of an organism which, in its very essence, is a unit. The biologist has long studied living organisms as wholes and will continue to do so with ever-increasing interest. But these studies can tell us nothing of the nature of the "physical basis of life," which no form of philosophy can ignore. It is for chemistry and physics to replace the vague concept "protoplasm"—a pure abstraction—by something more real and descriptive. I know of nothing which has shown that current efforts to this end do not deal with realities. It is only necessary for the biochemist to remember that his data gain their full significance only when he can relate them with the activities of the organism as a whole. He should be bold in experiment but cautious in his claims. His may not be the last word in the description of life, but without his help the last word will never be said.

## SOME ASPECTS OF BIOCHEMISTRY

### THE ORGANISING CAPACITIES OF SPECIFIC CATALYSTS

[Second Purser Memorial Lecture, 1932]

It was never my privilege to know personally the distinguished physiologist and clinician whom these lectures commemorate. I can yet feel great pride in delivering a lecture associated with his highly honoured name. It is for me a special pleasure to deliver it in this great centre of learning which, a few years ago, in conferring upon me one of its coveted Doctorates, did me an honour which I very deeply prize.

I will ask to be allowed to base my opening remarks on the theme of my predecessor's discourse. Sir Edward Sharpey-Schäfer dealt last year with the birth of a new physiology. In his view the revelation that organic functions in the animal body are controlled and co-ordinated by the circulation of endogenous chemical agencies so greatly changed the physiological field and outlook as to create a scientific discipline essentially new.

If we are entitled to make a distinction, based on diversity of methods and perhaps on some difference in standpoint, between classical physiology and modern biochemistry, it may be said that the revelation in question, while re-orientating thought in the former, certainly added justification to the aims and claims of the latter.

#### THE DETERMINANTS OF METABOLISM

In the last century descriptive physiology, and indeed biological science generally, gave little thought to the influence of internal molecular structure as one of the factors which determine the nature and behaviour of living systems. The progress of metabolism in these systems, with all the vital properties which it supports, was referred to the emergent properties of intact "protoplasm." These were hypothetical properties of a hypothetical entity; not susceptible to analysis, and not to be described in ordinary chemical language. Up to the end of the century there had been little effort, and, seemingly, on the part of many biologists, but little desire, to seek behind these assumed properties of protoplasm for specific determinants of metabolism. Materials suffer change in living systems because they are first built up, with loss of their own molecular identity, into a complex whole in which active change for some unexplained reason is inherent. Such was the mode of thought about metabolism which for a time was common and which has not wholly departed. It rejects analysis and leads to descriptions which are scientifically barren.

Realisation however of the specific activities of hormones—substances of known (or certainly ascertainable) and relatively simple chemical nature—was one of the happenings at about the turn of the century (others of

perhaps equal importance were nearly contemporaneous) which emphasised the need for recognising in living systems the patent influence of molecular structure, displayed in these as it is displayed everywhere in the field of organic chemistry.

The influence of each hormone (or, to employ Schäfer's more comprehensive and, I think, more logical term, of each autocoid) is as specific as it is profound. This specificity of action depends ultimately upon the characteristic molecular structure of each individual among these agents, and diversity of action among them depends upon diversity of structure. In the case of such relatively simple organic molecules specific properties can ultimately depend on nothing else but molecular structure; and to this there is a corollary. An organic molecule can display its properties only in an environment where it suffers or induces change. In a colloidal environment such as it will meet in the living cell it may, it is true, exert a powerful influence of a quite general sort; if, for instance, it be the molecule of an acid or base. When however its influence on its environment is highly specific it is sure that it meets, somewhere in the environment itself, with a display of properties, also due to specific molecular structure, which is adjusted to make relations with its own. The vague assumption of protoplasmic control avoided considerations such as these.

Research during the present century has, in the minds of many, replaced the conception of metabolism as comprising events determined by the obscure properties of a single biological entity (emergent properties lost with loss of its integrity) by such a view as the following. The living cell, at one definite level of its organisation, admitting that higher levels may be superimposed, is to be pictured as the seat of diverse but organised chemical reactions, in which substances identifiable by chemical methods undergo changes which can be followed by chemical methods. The molecules of these substances are activated and their reactions directed in space and time by the catalytic agencies which are commonly known as intracellular enzymes. The influence of these differs in no essentials from that of catalysts in non-living systems save that it is displayed in relations which are exceptionally specific. Their activating and directive influence is displayed mainly at surfaces or interfaces, of which some are the surfaces of colloid particles dispersed in an aqueous medium, while others are structural in a histological sense.

A conception of cell metabolism and its control such as that thus briefly indicated is now familiar; but it is, I think, justifiable and not untimely to attempt some appraisal of its intellectual worth. How far, if at all, does it carry us towards an acceptable description of living systems? Some answer to this question may be attempted.

#### INTRACELLULAR ENZYMES

The view in question is based upon the relatively recent recognition that a multiplicity of enzymes exists which have truly intracellular functions,

and are not destined merely to appear in secretions; further, upon the fact that these enzymes in isolation are found to catalyse *in vitro* reactions known to occur in the living cell; and lastly upon the justifiable faith that in the cell they must exert, though in complex relations, an influence identical with that observed outside it.

It is of historical and, I think, of psychological interest to remember that though the functions of digestive enzymes displayed outside the tissues had been so long familiar, the earliest suggestions that animal cells and tissue elements contain functional enzymes as an essential part of their constitution met with much scepticism. It was commonly urged up to near the end of the last century that though enzyme activity may be demonstrated in tissues *post mortem*, the fact is without bearing on the nature of metabolism. It means no more—such was a common view—than that the glands of digestion liberate, as it were accidentally, some small portion of their secretion into the blood stream. The inactive zymogens thus circulating are absorbed by the tissues, and, as an event without physiological significance, may appear as active enzymes on the death of the tissues. Even those who were prepared to admit that the enzymes in question were in some sense endogenous, held that their awakening to activity was wholly a *post mortem* phenomenon serving but to disintegrate dead tissues; true metabolic events they urged were due to “protoplasmic” and not enzymic activity. It was not remembered that in the case of any organism, including human society itself, activities which when disorganised make for disruption may be identical with those which when organised and disciplined are responsible for ordered progress. Death involves disorganisation and disorganisation death. Goethe emphasised this truth with a biological reference.

“Wer will was Lebendigs erkennen und beschreiben,  
Sucht erst den Geist herauszutreiben,  
Dann hat er die Teile in seiner Hand,  
Fehlt leider! nur das geistige Band.  
‘Encheiresin Naturae’ nennt’s die Chemie,  
Spottet ihrer Selbst, und weiss nicht wie.”

FAUST, sc. iv.

Although studies in comparative physiology had clearly demonstrated that intracellular digestion of food materials may certainly occur *in vivo*, it was long urged that even this was due to protoplasmic influence rather than to enzymic activity. It was not indeed until the turn of the century, when as you know it was proved that the alcoholic fermentation of sugar occurs under the influence of the expressed juice of yeast when it is entirely free from living cells, that conviction as to the functional importance of intracellular enzymes began to grow. Even then there was a desire on the part of many, though their view was soon disproved, to attribute the fermentation to “fragments” of living protoplasm suspended in the juice;

an illogical view for those with genuine holistic prejudices. Even as late as 1913 a prominent authority suggested that only a small part of the fermentation process is due to enzyme activity; the rest he thought depended on residual "vital" activity. It seems to me that much of this past opposition to more analytical views, though in a sense it foreshadowed the holistic philosophy now dominant, was due to no more than a kind of biological piety which held sacrosanct the conception of protoplasm as an entity.

### THE SPECIFIC ACTIVATION OF MOLECULES

I find I must employ the terms "catalysis," "activation" and "specificity" so frequently in the course of what follows that it may be well in parenthesis to comment upon them.

The familiar and convenient term catalysis as applied to the enormous acceleration of chemical reactions due to the mere presence of agencies which themselves remain intact when the course of change is complete, in no way describes or defines the actual mechanism which underlies the influence of such agencies. The term has long been applied to the phenomenon in complete ignorance of the underlying mechanism. Only recently indeed has intensive study, *inter alia* of the structure of surfaces, and of molecular orientation at surfaces, led to some knowledge concerning it. We may here be content to recognise that under the influence of a catalyst molecules become "activated"; so modified, that is, as to become more prone to undergo change. It is generally recognised that in any field of chemical change the molecules present at any moment are not all equally active; otherwise reactions would not follow their observed courses. Molecular activity may be increased, however, by a gain of energy due to external influences, to rise of temperature, to irradiation, and otherwise. In the promotion of activity by catalysis we are entitled from the available evidence to believe that the process is preceded by some kind of chemical union between the catalyst and the molecules concerned. As the result of such union a system is formed in which the affinity fields, or, we may say, the electron orbits within the molecule, are so modified that less energy need be supplied to establish that critical condition which results in activity. Thus in any given circumstances, at the temperature of the body, for instance, the progress of chemical change is greatly accelerated. That chemical union precedes activation in catalysis (and proof of this is perhaps especially clear in the case of catalysis by enzymes) offers an explanation for specificity in the phenomenon. It explains the circumstance that a given catalyst may be able to influence one particular molecule alone, or a very limited group of molecules. Aspects of structure in the activating agent must be adjusted to aspects of structure in the molecule to be activated before effective relations between them can be established. We may recall the classical analogy of lock and key as applied by Emil Fischer to the relations between enzyme and substrate.

## THE HIGH SPECIFICITY OF BIOLOGICAL CATALYSIS

You are doubtless aware that recent research with constantly improving technique is demonstrating that individual catalytic agencies in quite remarkable numbers and with diverse activities can be separated from animal tissue, or be shown to exist in them. It is common to apply the term enzyme to all of them, though a distinction to which I will refer is perhaps desirable. Hardest of study are those which can be obtained in colloidal solution and on these in particular an immense amount of valuable work has been done. Though perhaps no enzyme has been isolated many have been highly concentrated, yielding preparations of great activity. It will illustrate the efficiency of biological catalysis in general if we remember that an enzyme preparation may change in one second at room temperature anything from ten to a thousand times its weight of the substance with which it is in contact and to which it is specifically adjusted. Very many quantitative studies have been made of enzymes and their action, of the kinetics of reactions which they catalyse, of their varying affinities, of other conditions which affect their activities, and of the range of their individual influence. It is to the last that I wish to give special attention.

Specificity in relation to a substrate is not unknown as a feature of inorganic catalysis, but it dominates the biological field. An enzyme may display intense activity towards one special substrate, but none at all towards even the most closely related substances. Another may show a wider but still very limited range. Some may activate a group of homologous substances, but everywhere the range of activity is very narrow. Recognition of this limitation leads to what, for some, is an intellectual difficulty. So many and diverse are the reactions which may take place in an individual living cell that if each is specifically catalysed individual enzymes must be very numerous in a system which is very small. I have met those who therefore feel that our methods of study when applied to isolated enzymes must somehow introduce an element of falsity into the phenomena as observed. I cannot share that feeling. It may be, as I shall point out, that *in situ* a given catalytic mechanism may possibly extend its range beyond what is displayed in isolation, and it is logical that we should seek for signs of greater simplicity in the phenomena wherever they may be found. I feel myself, however, that the recognition of this high specificity among the agencies which control metabolism makes it possible for me to form some mental picture, however inadequate, of the extraordinary organisation among chemical events which living systems display. Without it I could form no such picture at all.

## BIOCHEMICAL REACTIONS

To the nature of the reactions which proceed under the influence of the agencies we are discussing I must spare but a few words. Though very various they fall into two main classes. The first comprises the reversible processes of hydrolysis and condensation suffered by a

multitude of molecules of various types. These reactions involve but small energy exchanges and are chiefly involved in the synthesis of complex molecules and structures in the cell balanced against the metabolic breakdown of these. They are controlled by enzymes which display relatively low affinity for their substrates. Reactions of the second class are those which yield energy for physiological work. They ultimately, in the majority of cases, result primarily in the oxidation of hydrogen by atmospheric oxygen. Recent research has added much new knowledge concerning the mechanism of these latter reactions and of the nature of their enzymic control. Here I must only remark that in almost every case the molecules to be oxidised, no less than the oxygen, must suffer activation before reaction occurs, and the former result is achieved by highly specialised enzymes. Thus even activated oxygen exerts no indiscriminate effects upon the cell contents. What shall be oxidised is a matter under complete enzymic control. The enzymes concerned show a high affinity for their substrates, a circumstance related with the energy changes involved. Another important characteristic of biological oxidations made clear during recent years is that transport of hydrogen atoms from molecule to molecule and ultimately to enzyme-activated oxygen is a dominant event in the reactions concerned. It has even been claimed, and perhaps justly, that all inspired oxygen unites with hydrogen alone; that which is expired in the carbon dioxide having its source in the oxygen of water. Progress in the study of the energy-yielding events in the cell has been greatly illuminating, but as the nature of the chemical reactions which underlie them is not of immediate importance to the theme of this lecture, I must not discuss them further.

### THE NATURE OF CELL CATALYSTS

In any endeavour to learn the precise nature of the catalytic agencies themselves we suffer from the circumstance that except in a few doubtful cases no one of them has yet been isolated with certainty as a chemical entity. It is almost certain indeed that they are incapable of isolation in a sense familiar to the chemist, and must elude ordinary chemical criteria of "purity." This because each, when active, is either associated with, or actually forms part of, a colloidal surface. It is true that in some cases we may be justified in the belief that the enzyme unit is of molecular magnitude and displays the properties of a molecule; but those who study enzymes have commonly come to the conclusion that such a molecule is maintained in stability and activity only when in association with a colloidal particle which acts so to speak as a carrier. Further, it is almost sure that in the majority of cases the unit catalytic structure is actually part of a surface; probably a small and localised part. In the case of the so-called soluble enzymes (enzymes as strictly defined) the surfaces are those of colloid particles. Other catalytic agencies proper to the cell are rather parts of a histological or structural surface.

The nature of what may be called structural catalysts is most easily illustrated by reference to the properties of the outer surfaces of bacteria displayed in contact with an external medium, and I would ask you to let me refer for a moment to these lower organisms. Some years ago it was shown by one who was then my colleague\* that the surface of *Bacillus coli* could catalytically activate the diverse molecules of no less than 56 substances out of 105 tested by adding them to the medium. They were activated in the sense that they became donators or acceptors of hydrogen (oxidised that is, or reduced) though in the conditions of experiment quite stable in the absence of the organism. It should be noted that though these catalytic powers are exerted by the living cell many of them remain, and can then be most conveniently studied, when cell growth is prevented and even when the organism is no longer alive. Now, considerations which I must not stop to discuss have shown that on such a surface complete specificity of relation between catalyst and the molecule activated may not hold; we need not perhaps assume that the activation of fifty diverse substrates calls for as many as fifty diverse catalytic agencies. Nevertheless, specificity still dominates the phenomena. It would seem sure that at very many localities on the surface of an individual bacillus there are separate localised areas each with structural peculiarities of its own and these determine its specific catalytic activities.

You should realise how closely such a state of affairs is paralleled in inorganic phenomena.† It has been shown in the case of catalysis by metals that only localised portions of the metallic surface, where for this or that reason the arrangement of the atoms is peculiar, are catalytically active. In other cases it is found that the preservation of an active surface is much assisted by depositing the catalyst on some entirely inactive supporting material which serves as a carrier with functions not unlike those of the colloidal carrier associated with a soluble enzyme. The peculiar structure necessary for activity is thereby stabilised. Especially, however, in the effects of substances which destroy, reversibly or irreversibly, the catalytic powers of this or that active surface—so-called “poisons”—are analogies between the biological surface and a metal surface illustrated. Owing to varying sensibility to this or that “poison,” which itself depends upon niceties in the active structure, a surface region which activates one molecule or one type of molecule may be thrown out of action, while those activating other molecules may be left with their influence intact. This has been shown with respect to bacillary surfaces and metallic surfaces alike. In the case of the former the method has yielded much information as to the properties of different catalytic patches on the cell wall. If the bacillary structure be destroyed, as it is under the influence of the interesting agent lysozyme, it is noteworthy that certain catalytic mechanisms disappear while others are left intact. The former are just those which we should expect to find

\* J. H. Quastel, *Biochem. Journ.* 1926, **20**, 166.

† For literature, see N. K. Adam, *Physics and Chemistry of Surfaces*, Oxford, 1931

active at the cell surface. While the nature of the bacillary organism makes such studies relatively easy we may legitimately assume, and there are facts to justify the assumption, that analogous conditions obtain on surfaces, external and internal, of tissue cells in general.

Before leaving this brief consideration of the catalytic powers of bacteria, I must tell you of an observation recently made by two of my colleagues at Cambridge.\*

I have mentioned that a large proportion of the catalytic powers of bacillary surfaces remain intact when the organism has ceased to grow. A culture may be washed and suspended in a medium which cannot support growth but the then quiescent cells still promote chemical reactions in that medium.

*Bacillus coli*, when growing, can activate the molecules of formic acid. When this substance is added to its nutritive medium the organism brings about its decomposition into carbon dioxide and free hydrogen. A culture having grown in the presence of formic acid, and displaying this activity, may be removed, washed and transferred to a non-nutritive medium also containing formic acid. Though there is then no growth at all, the acid is very actively decomposed as before. If, however, the organism has been previously grown on ordinary media free from formates, then the washed quiescent cells show no trace of such activity. Note that the only varied factor determining this striking difference, which is constantly observed in such experiments, is the presence or absence of the formic acid during growth.

We must interpret these results in some way as the following. During the progress of growth and division the organism is plastic, and the newly formed cells register, in some aspect of their structure, an impress due to contact with formic acid molecules. Thus, in manner frankly mysterious, is established a new catalytic mechanism. It is retained when growth ceases, having become a permanent feature of the cell structure; but it cannot be directly impressed upon quiescent cells. How contact with a particular molecule can result in the new formation of catalyst specifically adjusted to activate that molecule is a question at present without an intelligible answer. I believe, however, that the phenomenon, which, though exceptionally clear in the case I have discussed, is paralleled in other cases, is of great biological significance. We do not yet know how widely its suggestions may carry, but it is more than likely that growing cells, even the cells of an animal embryo, may develop some of their enzymes under compulsion from specific molecules in their environment. One thing seems surely indicated; an enzyme may arise in a cell not by the synthesis of an entirely new molecule, but by an alteration in the configuration of existing materials. If this can be accepted, it will be found to simplify thought concerning that continuity of enzyme equipment which is observed when cells divide.

\* M. Stephenson & L. H. Stickland, *Biochem. Journ.* 1933, **27**, 1528.

## ORGANIZED EVENTS IN TISSUE EXTRACTS

Before I ask you to decide how far the considerations which have been before you can give any acceptable help towards an understanding of the remarkable organization of chemical events displayed in the living cell, I want to point out that chemical organization at no low level may be displayed even in mere extracts from cells or tissues. It is important to my theme that this should be recognized. Consider for instance the reactions which proceed in a simple aqueous or saline extract from muscle-tissue when made with certain well understood precautions.\* I recently made such a preparation for the purpose of this reference. Superficially examined this particular preparation would have been described merely as a colloidal solution entirely free from cell structure containing 1.8 per cent. of total solids, of which 1.2 per cent. was protein dispersed in a lyophil condition with an average isoelectric point at about pH 6. It contained phosphates, some free and some in organic combination, a little creatin and other extractives in small amount. A description like this, however, would be entirely inadequate. For such an extract when examined more closely is found to be the seat of numerous chemical reactions which progress from the moment of its preparation. Of these some of the most prominent proceed together so as to result in the conversion of carbohydrate into lactic acid, an event so highly characteristic of metabolism in intact muscle. It is easy to show that the progress of these reactions depends on the presence of a set of enzymes with specific activities.

In such an extract various carbohydrates are converted into lactic acid, but our attention may be confined to glycogen and glucose. Glycogen is first hydrolysed by an amylolytic enzyme yielding sugars or six-carbon derivatives which are then under the influence of an esterifying enzyme, combined with phosphoric acid. The least stable of the phosphoric esters so formed is then decomposed by a catalyst which may be called the muscle symase, yielding lactic acid and liberating the phosphoric acid. There is, however, increasing probability that a 3-carbon precursor of lactic acid first appears from the ester, namely methylglyoxal. A special enzyme, glyoxalase, which can be shown to exist in our extract, then determines the final conversion of this into lactic acid. That these successive processes depend upon thermolabile enzymes is sure; each step can be isolated and studied separately. There is, however, a further complication. The production of lactic acid does not occur in the extract without the intervention of a factor which is not itself an enzyme, but is of the class of "co-enzymes." I have hitherto said nothing about agencies of this sort, though many such are known. In certain cases activation does not proceed when enzyme and substrate are in contact by themselves; a *tertium quid*, a co-enzyme, is necessary to complete the system. This circumstance we may believe plays its own part in the organisation of events in the cell.

\* O. Meyerhof, *Biochem. Zeitsch.*, 1926, 178, 395, 462; *Ibid.*, 1927, 183, 176.

Analogous relations are found in the domain of inorganic catalysis. In the muscle system the nature of the co-agent is now precisely known.\* In chemical language it is an adenylyl pyrophosphoric acid, the highly specific properties of which add much of interest to the phenomena we are considering.

There is, however, yet more complexity in the events which proceed in our muscle extract. Unless these events are studied when the extract is quite freshly made it is noteworthy that while the complex molecule of glycogen is readily converted into lactic acid, the more immediately related glucose molecule unexpectedly is not. The reason of this would seem to be that an active form of glucose is necessary for the indispensable step of esterification with phosphoric acid. Such an active form is liberated during the hydrolysis of the glycogen and immediately esterified before assuming the more ordinary stable form of the sugar. However this may be, it is a fact that yet another agent is present in fresh extracts, and therefore doubtless in the muscle itself. This is capable of activating ordinary glucose which is then rapidly converted into lactic acid. This agent, which is known as hexokinase, is exceedingly unstable and rapidly disappears from an extract after it is made. It should be noted here that chemical events quite other than those connected with lactic acid production occur in our extract, and there is no mutual interference. This independence is due in part to the nature of the molecules concerned, but still more to the fact that distinct enzymes each with a specific action are in control.

We may recall also here that other familiar case in which chemical organisation is shown to persist in cell extracts wholly free from all structure. I allude once more to alcoholic fermentation as induced by yeast juice, or by extracts from the yeast cell. We know that the juice or extract contains a whole battery of enzymes each of which plays a special and necessary part in controlling the successive processes which finally result in the production of alcohol and carbon dioxide.

In such cell extracts chemical organisation is very clearly displayed, for while successive reactions are involved the end result is only reached when these proceed in due and proper order. It will be clear to you, I think, on consideration, that this ordered progress is secured because the activities of the enzymes are specific. No one of the reactions occurs spontaneously; all must be catalysed. No change, for instance, occurs in the original carbohydrate molecules, save when in traversing the aqueous medium they meet the proper enzyme surface. Only when the second adjusted enzyme is so met do the molecules produced in the first reaction suffer the change which necessarily comes next in succession; and so on throughout the series of reactions. The due and necessary order in series is thus inevitably secured. The kinetic energy of the various molecular species secures their distribution through the aqueous medium, and the frequency with which each species meets with the enzyme adjusted to its structure

\* K. Lohmann, *Naturwiss.*, 1929, 17, 624; *Biochem. Zeitsch.*, 1931, 237, 455.

(others will have no effect) will decide the velocity with which it will undergo change, and this, of course, depends upon the concentration of each enzyme species present. It is true that in such extracts the normal velocity of reactions will usually not be maintained. It is also true that the nice adjustment of reaction velocities, both absolute and relative, must be a fundamental happening in the living system. The one feature of phenomena within a living system which is certainly metrical is velocity of change. It is a mistake to suppose, however, that the progress and velocity of reactions can never be followed in living tissues. The old taunt that chemical studies of living matter must be futile since the moment they are applied life ceases, has but little force. Let me remind you only of one method out of many by which the feature of this objection is avoided. When living material is known to be uniform, so that the condition of a sample at any moment represents the condition of the whole, it is easy while a given reaction is proceeding to remove such samples at successive points along a time scale. Progress of change in each successive sample can be instantaneously ascertained, and estimations of this or that product of change duly made. The velocity curve of reactions may thus be obtained, though they have actually occurred in the living tissue. Studies of this kind supplemented by equally quantitative studies in tissue extracts, as well as other methods, thus make it possible to follow such dynamic events. Only of recent years, I think, will suggest the data so obtained are unreal or incapable of application to the living cell itself.

#### ORGANIZATION IN THE CELL

Let us now consider the case of any individual cell in due relations with its environment, whether an internal environment as in the case of the tissue cells of higher animals, or an external environment as in the case of unicellular organisms. Materials for maintenance of the cell enter it from the environment. In the proper selection of such materials permeability questions are involved, but of deeper significance in that selection is the specificity of the cell catalysts. It has often been said that the living cell differs from all other systems in its power of selecting from a heterogeneous environment the right material for the maintenance of its structure; but it is no vital act but the nature of its specific catalysts which determine what it "selects." If a molecule gains entry into the cell and meets no catalytic influence capable of activating it, save for osmotic adjustments, nothing further happens. Any molecule which does meet an adjusted enzyme cannot fail to suffer change and become directed into some one of the paths of metabolism. It must be, moreover, remembered here that enzymes as specific catalysts not only promote reactions, but determine their direction. We have just seen, for example, that the glucose molecule, though its inherent chemical potentialities are, of course, always the same, is converted into lactic acid by one enzyme system, but into alcohol and carbon dioxide by another. If it be syntheses in the cell which are most

With regard, moreover, to the special vital characteristics of the cell just mentioned, I feel at least entitled to point out that even in the region of embryological development and in the phenomena of genetics, modern research has shown that chemical aspects intrude to an extent not earlier recognised, and instances of this are frequently coming to light. I must be content with a single example to illustrate what I mean, and will choose the case of "organisators" in the embryo.\* Some will remember that in a developing vertebrate embryo, although at its earlier stages the individual cells possess the power of giving rise to almost any embryonic structure, depending for their fate merely on the position in which they are found or artificially placed, there comes a moment when their ultimate fate is henceforth predetermined. This is at the stage of gastrulation. It has been shown that this change in potentiality is due to a remarkable influence exerted upon the rest of the embryo by a specialised group of cells situated in the region of the dorsal lip of the blastopore. This group constitutes the *organiser* or organisator. If it be removed, normal differentiation ceases. On the other hand, if it be grafted into another gastrula it will compel cells in the host, which without its influence might have quite another fate, to develop, in its neighbourhood and in abnormal positions, the primary axial organs, namely, a neural plate, notochord and somites. An influence so remarkable and so subtle might well tempt to the belief that it is transmitted to the responsive cells by other than material modes. Yet it has been shown that the structure of the organiser may be destroyed by grinding or crushing without losing its power of controlling the fate of other cells. I find that most who are studying phenomena, of which this is but an example, are looking to chemical transmission; to something like hormonal influence. We are indeed not yet sure where chemical organisation may end. The conception of organisation at different levels is doubtless, in a sense, an abstraction. It need, however, lead to no false views concerning the cell as a whole, and in my belief it can greatly aid in any description of a living system which is within the compass of biochemistry.

This leads me to recur to my opening remarks. The influence of hormones in the body is surely exerted upon the cells of tissues at the chemical level of organisation. The special chemical structure of each one asserts itself amid the interplay of chemical events, perhaps modifying catalytic activities, perhaps exercising catalytic properties of its own, thus modifying the course or velocity of reactions. Such modifications become then responsible for the physiological effects which we observe. I may add in parenthesis that the same considerations may well apply to the highly specific functions of vitamins. It is indeed, I think, illogical at present to make too sharp a distinction between these two groups of active agencies merely because one group is endogenous and the other exogenous.

It is highly satisfactory to realise the remarkable progress which is being

\* Cf. H. Spemann, *Arch. f. Entwicklungsmechan.*, 1918, 43, 448; *Naturwiss.*, 1927, 15, 946.

made in the constitutional chemistry of vitamins and of the more recently discovered hormones. It would seem that we shall not have long to wait before the molecular structure of the majority is known. It thus becomes an immediate and essential task of the biochemist to decide precisely where, and, in chemical terms, how, the specific structure of each exerts its powerful influence; a task of difficulty, but one not outside the reach of experiment, and one within the category of legitimate aims.

In the course of such endeavours we shall certainly increase our knowledge of chemical organisation in the tissues, and, if I mistake not, obtain further confidence in the organising powers of specific catalysis. To this end studies of isolated parts of the organism will certainly continue, and I must not longer avoid definite reference to the circumstance that, in some quarters at least, such studies of parts in isolation remain under suspicion. Their significance is chemical alone, not biological. Such is the opinion of at least a few.

#### BIOCHEMICAL METHODS AND THE PHILOSOPHY OF "WHOLE"

It is impossible to discuss the nature of life from any standpoint without making contacts with philosophical views, and meeting, it may be, with weighty criticism. As you know, a standpoint which General Smuts has called Holism is just now assumed by many writers. We are frequently reminded that "a whole is not merely a sum of parts or constituted by its parts. Its nature lies in its constitution more than its parts. The part in the whole is no longer the same as the part in isolation." It would seem, indeed, that almost undue emphasis is being placed upon considerations which even common sense recognises up to a point and within the limits of its powers. But it is sure that so far as science is concerned illuminating knowledge of wholes has been gained by the study of parts. The study of wholes in biology usually demonstrates highly interesting and significant, but unexplained, behaviour. All that experimental biochemistry to-day need really ask from philosophy is a decision as to whether its study of parts, with methods akin to the methods of scientific inquiry elsewhere, yields data which when applied in pictorial thought to the whole living organism can result in conceptions which are in any sense false. I am not sure that the experimentalist himself is not as well qualified to judge of this as some among philosophers.

There is indeed one distinguished experimentalist who being also a philosopher has for many years vigorously insisted that any attempt whatever to describe living organisms in terms of chemistry and physics must be futile. I must not here attempt any appraisal of Dr. J. S. Haldane's standpoint. Indeed, after some years of respectful effort I cannot pretend to understand it completely. I must be content with a single quotation from his writings which will suffice to illustrate the difficulties I personally find in his teaching. In his Gifford Lectures, delivered in 1927-28, after discussing the extraordinary delicacy with which the respiratory centre

responds to minute changes in the hydrogen-ion concentration in the blood—resulting in a fascinating physiological co-ordination which, as you know, was finally established by Dr. Haldane's own brilliant experiments—he remarks: "If we ask why the centre should act more vigorously or become quiescent according as the hydrogen-ion pressure rises or falls, to an extent so minute that it can hardly be detected by physical or chemical means, *there is no physical or chemical answer*" (the italics are mine).

It is hard to accept wholly *a priori* assertions of this kind. Are we, for example, entirely to ignore the familiar circumstances that while all colloidal systems are highly sensitive to changes in hydrogen-ion concentration, some, owing to special conditions, are much more sensitive than others? Present knowledge, it is true, is not adequate to account for the peculiar sensitiveness of the cells in the respiratory centre, but the belief is surely justified that modern colloid chemistry will prove capable of accounting for it. To accept intellectual help from such physico-chemical considerations when thinking of one particular aspect of co-ordination in the body does not prevent us from remembering and respecting the unity of the body as a whole. Dr. Haldane's teaching, however, seems always to imply that the use of such intellectual help leads to a false conception of the organism, though it is hard to understand precisely why. For him the task of the physiologist is to accept the organism as axiomatic, and to illustrate by his experiments with increasing clearness how perfect are its adjustments. This is a task of great interest and importance though perhaps apt to lead to *a priori* assumptions; but if it be its final task physiology in its aims stands apart from other branches of science. That it should so stand has indeed long been Dr. Haldane's contention. He now feels, however, that biology and the physical sciences are coming together because the latter have discovered that they too have to study "organisms"; "biological" methods and thought must therefore triumph. Is it not true nevertheless that in modern chemistry or physics the enlightening knowledge of wholes has come with increasing ability to study the nature of parts? Surely this is true, alike of the atom and the molecule.\* Why not of the living organism?

As bearing on such questions I would like, in closing my remarks, to call your attention to a few brief extracts from the writings of Professor A. N. Whitehead. I have often endeavoured to appraise the precise attitude of this profound thinker towards the efforts of chemistry to throw light on the nature of living stuff by studies of its parts, and though his teaching emphasises, of course, the uniqueness of wholes, I conclude that he at least justifies those efforts. You are aware that in the world-picture as viewed from Dr. Whitehead's standpoint, which he calls that of organic mechanism, one sees underlying realities not as entities displaying only *external* relations, like the atoms of Leucippus and Democritus or even the atoms of Dalton which possessed more character, but as entities with a set of *internal* relations which are of their very essence. In this sense they are organisms. The

\* *The Sciences and Philosophy*, 1928, p. 88.

unit entities of Newtonian science are pure abstractions; concrete fact is represented by organisms. Such is Whitehead's standpoint. Modern physics has, of course, convinced us all that this applies to the atom, and we have long known that internal relations stamp the nature of the chemical molecule and especially that of organic molecules. But for Dr. Whitehead the conception of the organism is all-embracing, and needless to say, therefore, his teaching emphasises the subservience of the part to the whole. Although a mathematician he seems to attach much importance to the study of living systems, as especially deserving the attention of philosophy, and in one of his books he makes remarks directly bearing on the question before us.\* He writes as follows:

"The relation of part to whole has the special reciprocity associated with the notion of organism, in which the part is for the whole; but this relation reigns throughout nature and does not start with the special case of the higher organisms.

"Further, viewing the question as a matter of chemistry, there is no need to construe the actions of each molecule in a living body by its exclusive particular reference to the pattern of the complete living organism. It is true that each molecule is affected by the aspect of this pattern as mirrored in it, so as to be otherwise than what it would have been if placed elsewhere. In the same way, under some circumstances an electron may be a sphere, and under other circumstances an egg-shaped volume. The mode of approach to the problem, so far as science is concerned, is merely to ask if molecules exhibit in living bodies properties which are not to be observed amid inorganic surroundings.

"It would, however, be entirely in consonance with the empirically observed action of environments if the direct effects of aspects as between the whole body and its parts were negligible.

"Thus the question for physiology is the question of the physics of molecules in cells of different characters."

Dr. Whitehead's views cannot, of course, be adequately presented in brief detached quotations, but if the application of chemical methods to the study of life needs more moral support from philosophy than do other scientific pursuits, I think it finds a charter in his writings.

Biochemistry has many new tasks ahead, but it must continue its concern with the *organising* potentialities of specific catalysis. These potentialities have hitherto lacked adequate appraisal in chemical thought.

I will close this lecture with final emphasis on my theme. I will ask you to consider whether catalysis on highly specific lines is not among the most fundamental and significant phenomena in nature, and whether as displayed amid the complexities of the cell it is not as truly an essential attribute of life as any other physical attribute whatever.

\* *Science and the Modern World.*

## SOME CHEMICAL ASPECTS OF LIFE

[*Rep. Brit. Ass.* 1933, p. 1]

## I

THE British Association returns to Leicester with assurance of a welcome as warm as that received twenty-six years ago, and of hospitality as generous. The renewed invitation and the ready acceptance speak of mutual appreciation born of the earlier experience. Hosts and guests have to-day reasons for mutual congratulations. The Association on its second visit finds Leicester altered in important ways. It comes now to a city duly chartered and the seat of a bishopric. It finds there a centre of learning, many fine buildings which did not exist on the occasion of the first visit, and many other evidences of civic enterprise. The citizens of Leicester on the other hand will know that since they last entertained it the Association has celebrated its centenary, has four times visited distant parts of the Empire, and has maintained unabated through the years its useful and important activities.

In 1906 the occupant of the presidential chair was, as you know, Sir David Gill, the eminent astronomer who, unhappily, like many who listened to his address, is with us no more. Sir David dealt in that address with aspects of science characterised by the use of very exact measurement. The exactitude which he prized and praised has since been developed by modern physics and is now so great that its methods have real aesthetic beauty. In contrast I have to deal with a branch of experimental science which, because it is concerned with living organisms, is in respect of measurement on a different plane. Of the very essence of biological systems is an ineludable complexity, and exact measurement calls for conditions here unattainable. Many may think, indeed, though I am not claiming it here, that in studying life we soon meet with aspects which are non-metrical. I would have you believe, however, that the data of modern biochemistry, which will be the subject of my remarks, were won by quantitative methods fully adequate to justify the claims based upon them.

Though speculations concerning the origin of life have given intellectual pleasure to many, all that we yet know about it is that we know nothing. Sir James Jeans once suggested, though not with conviction, that it might be a disease of matter—a disease of its old age! Most biologists, I think, having agreed that life's advent was at once the most improbable and the most significant event in the history of the universe, are content for the present to leave the matter there.

We must recognise, however, that life has one attribute that is fundamental. Whenever and wherever it appears the steady increase of entropy displayed

by all the rest of the universe is then and there arrested. There is no good evidence that in any of its manifestations life evades the second law of thermodynamics, but in the downward course of the energy-flow it interposes a barrier and dams up a reservoir which provides potential for its own remarkable activities. The arrest of energy degradation in living nature is indeed a primary biological concept. Related to it, and of equal importance, is the concept of organisation.

It is almost impossible to avoid thinking and talking of life in this abstract way, but we perceive it, of course, only as manifested in organised material systems, and it is in them we must seek the mechanisms which arrest the fall of energy. Evolution has established division of labour here. From far back, the wonderfully efficient functioning of structures containing chlorophyll has, as everyone knows, provided the trap which arrests and transforms radiant energy—fated otherwise to degrade—and so provides power for nearly the whole living world. It is impossible to believe, however, that such a complex mechanism was associated with life's earliest stages. Feasting organisms illustrate what was perhaps an earlier method. The so-called autotrophic bacteria obtain energy for growth by the catalysed oxidation of material belonging wholly to the inorganic world; such as sulphur, iron or ammonia, and even free hydrogen. These organisms dispense with solar energy, but they have lost in the evolutionary race because their method lacks economy. Other existing organisms, certain purple bacteria, seem to have taken a step towards greater economy, without reaching that of the green cell. They dispense with free oxygen and yet obtain energy from the inorganic world. They control a process in which carbon dioxide is reduced and hydrogen sulphide simultaneously oxidised. The molecules of the former are activated by solar energy which their pigmentary equipment enables these organisms to arrest.

Are we to believe that life still exists in association with systems that are much more simply organised than any bacterial cell? The very minute filter-passing viruses which, owing to their causal relations with disease, are now the subject of intense study, awaken deep curiosity with respect to this question. We cannot yet claim to know whether or not they are living organisms. In some sense they grow and multiply, but, so far as we yet know with certainty, only when inhabitants of living cells. If they are nevertheless living, this would suggest that they have no independent power of obtaining energy and so cannot represent for us the earliest forms in which life appeared. At present, however, judgment on their biological significance must be suspended. The fullest understanding of all the methods by which energy may be acquired for life's process is much to be desired.

In any case every living unit is a transformer of energy however acquired, and the science of biochemistry is deeply concerned with these transformations. It is with aspects of that science that I am to deal and if to them I devote much of my address my excuse is that since it became a major branch

of inquiry, biochemistry has had no exponent in the chair I am fortunate enough to occupy.

As a progressive scientific discipline it belongs to the present century. From the experimental physiologists of the last century it obtained a charter, and, from a few pioneers of its own, a promise of success; but for the furtherance of its essential aim that century left it but a small inheritance of facts and methods. *By its essential or ultimate aim I myself mean an adequate and acceptable description of molecular dynamics in living cells and tissues.*

## II

When this Association began its history in 1831 the first artificial synthesis of a biological product was, as you will remember, but three years old. Primitive faith in a boundary between the organic and the inorganic which could never be crossed, was only just then realising that its foundations were gone. Since then, during the century of its existence, the Association has seen the pendulum swing back and forth between frank physico-chemical conceptions of life and various modifications of vitalism. It is characteristic of the present position and spirit of science that sounds of the long conflict between mechanists and vitalists are just now seldom heard. It would almost seem, indeed, that tired of fighting in a misty atmosphere each has retired to his tent to await with wisdom the light of further knowledge. Perhaps, however, they are returning to the fight disguised as determinist and indeterminist respectively. If so, the outcome will be of great interest. In any case I feel fortunate in a belief that what I have to say will not, if rightly appraised, raise the old issues. To claim, as I am to claim, that a description of its active chemical aspects must contribute to any adequate description of life, is not to imply that a living organism is no more than a physico-chemical system. It implies that at a definite and recognisable level of its dynamic organisation an organism can be logically described in physico-chemical terms alone. At such a level indeed we may hope ultimately to arrive at a description which is complete in itself, just as descriptions at the morphological level of organisation may be complete in themselves. There may be yet higher levels calling for discussion in quite different terms.

I wish, however, to remind you of a mode of thought concerning the material basis of life, which though it prevailed when physico-chemical interpretations were fashionable, was yet almost as inhibitory to productive chemical thought and study as any of the claims of vitalism. This was the conception of that material basis as a single entity, as a definite though highly complex chemical compound. Up to the end of the last century and even later the term "protoplasm" suggested such an entity to many minds. In his brilliant presidential address at the Association's meeting at Dundee twenty-two years ago, Sir Edward Sharpey-Schäfer, after remarking that the elements composing living substances are few in number, went



LEICESTER LECTURE, 1933.

"By the essential and ultimate aim of biochemistry I mean an adequate and acceptable description of molecular dynamics in living cells and tissues. . . ." (p. 244).





when it issues united with carbon as carbonic acid. The whole mystery of life lies hidden in that process, and for the present we must be content with simply knowing the beginning and the end." What we feel entitled to say to-day concerning the respiration of muscle and of the events associated with its activity requires, as I have suggested, a different language, and for those not interested in technical chemical aspects the very change of language may yet be significant. The conception of continuous building up and continuous breakdown of the muscle substance as a whole, has but a small element of truth. The colloidal muscle structure is, so to speak, an apparatus, relatively stable even as a whole when metabolism is normal, and in essential parts very stable. The chemical reactions which occur in that apparatus have been followed with a completeness which is, I think, striking. It is carbohydrate stores distinct from the apparatus (and in certain circumstances also fat stores) which undergo steady oxidation and are the ultimate sources of energy for muscular work. Essential among successive stages in the chemical breakdown of carbohydrate which necessarily precede oxidation is the intermediate combination of a sugar (a hexose) with phosphoric acid to form an ester. This happening is indispensable for the progress of the next stage, namely the production of lactic acid from the sugar, which is an anaerobic process. The precise happenings to the hexose sugar while in combination with phosphoric acid are from a chemical standpoint remarkable. Very briefly stated they are these. One half of the sugar molecule is converted into a molecule of glycerin and the other half into one of pyruvic acid. Now with loss of two hydrogen atoms glycerin yields lactic acid, and, with a gain of the same, pyruvic acid also yields lactic acid. The actual happening then is that hydrogen is transferred from the glycerin molecule while still combined with phosphoric acid to the pyruvic acid molecule with the result that two molecules of lactic acid are formed.\* The lactic acid is then, during a cycle of change which I must not stop to discuss, oxidised to yield the energy required by the muscle.

But the energy from this oxidation is by no means directly available for the mechanical act of contraction. The oxidation occurs indeed after and not before or during a contraction. The energy it liberates secures however the endothermic resynthesis of a substance, creatin phosphate, of which the breakdown at an earlier stage in the sequence of events is the more immediate source of energy for contraction. Even more complicated are these chemical relations, for it would seem that in the transference of energy from its source in the oxidation of carbohydrate to the system which synthesises creatin phosphate, yet another reaction intervenes, namely, the alternating breakdown and resynthesis of the substance adenylyl pyrophosphate. The sequence of these chemical reactions in muscle has been followed and their relation in time to the phases of contraction and relaxation is established. The means by which energy is transferred from one reacting

\* Lecture by Otto Meyerhof. In the press (*see Nature*).



it not equipped with catalysts every living unit would be a static system.

With the phenomena of catalysis I will assume you have general acquaintance. You know that a catalyst is an agent which plays only a temporary part in chemical events which it nevertheless determines and controls. It reappears unaltered when the events are completed. The phenomena of catalysis, though first recognised early in the last century, entered but little into chemical thought or enterprise, till only a few years ago they were shown to have great importance for industry. Yet catalysis is one of the most significant devices of nature, since it has endowed living systems with their fundamental character as transformers of energy, and all evidence suggests that it must have played an indispensable part in the living universe from the earliest stages of evolution.

The catalysts of a living cell are the enzymic structures which display their influences at the surface of colloidal particles or at other surfaces within the cell. Current research continues to add to the great number of these enzymes which can be separated from, or recognised in, living cells and tissues, and to increase our knowledge of their individual functions.

A molecule within the system of the cell may remain in an inactive state and enter into no reactions until at one such surface it comes in contact with an enzymic structure which displays certain adjustments to its own structure. While in such association the inactive molecule becomes (to use a current term) "activated," and then enters on some definite path of change. The one aspect of enzymic catalysis which for the sake of my theme I wish to emphasise is its high specificity. An enzyme is in general adjusted to come into effective relations with one kind of molecule only, or at most with molecules closely related in their structure. Evidence based on kinetics justifies the belief that some sort of chemical combination between enzyme and related molecule precedes the activation of the latter, and for such combinations there must be close correlation in structure. Many will remember that long ago Emil Fischer recognised that enzymic action distinguishes even between two optical isomers and spoke of the necessary relation being as close as that of key and lock.

There is an important consequence of this high specificity in biological catalysis to which I will direct your special attention. A living cell is the seat of a multitude of reactions, and in order that it should retain in a given environment its individual identity as an organism, these reactions must be highly organised. They must be of determined nature and proceed mutually adjusted with respect to velocity, sequence, and in all other relations. They must be in dynamic equilibrium as a whole and must return to it after disturbance. Now if of any group of catalysts, such as are found in the equipment of a cell, each one exerts limited and highly specific influence, this very specificity must be a potent factor in making for organisation.

Consider the case of any individual cell in due relations with its environment, whether an internal environment as in the case of the tissue cells of

higher animals, or an external environment as the case of unicellular organisms. Materials for maintenance of the cell enter it from the environment. Discrimination among such materials is primarily determined by permeability relations, but of deeper significance in that selection is the specificity of the cell catalysts. It has often been said that the living cell differs from all non-living systems in its power of selecting from a heterogeneous environment the right material for the maintenance of its structure and activities. It is, however, no vital act but the nature of its specific catalysts which determines what it effectively "selects." If a molecule gains entry into the cell and meets no catalytic influence capable of activating it, nothing further happens save for certain ionic and osmotic adjustments. Any molecule which does meet an adjusted enzyme cannot fail to suffer change and become directed into some one of the paths of metabolism. It must here be remembered, moreover, that enzymes as specific catalysts not only promote reactions, but determine their direction. The glucose molecule, for example, though its inherent chemical potentialities are, of course, always the same, is converted into lactic acid by an enzyme system in muscle but into alcohol and carbon dioxide by another in the yeast cell. It is important to realise that diverse enzymes may act in succession and that specific catalysis has directive as well as selective powers. If it be syntheses in the cell which are most difficult to picture on such lines, we may remember that biological syntheses can be, and are, promoted by enzymes, and there are sufficient facts to justify the belief that a chain of specific enzymes can direct a complex synthesis along lines predetermined by the nature of the enzymes themselves. I should like to develop this aspect of the subject even further, but to do so might tax your patience. I should add that enzyme-control, though so important, is not the sole determinant of chemical organisation in a cell. Other aspects of its colloidal structure play their part.

### III

It is surely at that level of organisation, which is based on the exact co-ordination of a multitude of chemical events within it, that a living cell displays its peculiar sensitiveness to the influence of molecules of special nature when these enter it from without. The nature of very many organic molecules is such that they may enter a cell and exert no effect. Those proper to metabolism follow, of course, the normal paths of change. Some few, on the other hand, influence the cell in very special ways. When such influence is highly specific in kind it means that some element of structure in the entrant molecule is adjusted to meet an aspect of molecule structure somewhere in the cell itself. We can easily understand that in a system so minute the intrusion of a few such molecules may so modify existing equilibria as to affect profoundly the observed behaviour of the cell.

Such relations, though by no means confined to them, reach their

and co-ordinate events in the animal body by virtue of their specific molecular structure, it is well not to separate too widely in thought the functions of hormones from those of vitamins. Together they form a large group of substances of which every one exerts upon physiological events its own indispensable chemical influence.

Hormones are produced in the body itself, while vitamins must be supplied in the diet. Such a distinction is, in general, justified. We meet occasionally, however, an animal species able to dispense with an external supply of this or that vitamin. Evidence shows, however, that individuals of that species, unlike most animals, can in the course of their metabolism synthesise for themselves the vitamin in question. The vitamin then becomes a hormone. In practice the distinction may be of great importance, but for an understanding of metabolism the functions of these substances are of more significance than their origin.

The present activity of research in the field of vitamins is prodigious. The output of published papers dealing with original investigations in the field has reached nearly a thousand in a single year. Each of the vitamins at present known is receiving the attention of numerous observers in respect both of its chemical and biological properties, and though many publications deal, of course, with matters of detail, the accumulation of significant facts is growing fast.

It is clear that I can cover but little ground in any reference to this wide field of knowledge. Some aspects of its development have been interesting enough. The familiar circumstance that attention was drawn to the existence of one vitamin ( $B_1$  so called) because populations in the East took to eating milled rice instead of the whole grain; the gradual growth of evidence which links the physiological activities of another vitamin (D) with the influence of solar radiation on the body, and has shown that they are thus related, because rays of definite wavelength convert an inactive precursor into the active vitamin, alike when acting on foodstuffs or on the surface of the living body; the fact again that the recent isolation of vitamin C, and the accumulation of evidence for its nature started from the observation that the cortex of the adrenal gland displayed strongly reducing properties; or yet again the proof that a yellow pigment widely distributed among plants, while not the vitamin itself, can be converted within the body into vitamin A; these and other aspects of vitamin studies will stand out as interesting chapters in the story of scientific investigation.

In this very brief discussion of hormones and vitamins I have so far referred only to their functions as manifested in the animal body. Kindred substances, exerting analogous functions are, however, of wide and perhaps of quite general biological importance. It is certain that many micro-organisms require a supply of vitamin-like substances for the promotion of growth, and recent research of a very interesting kind has demonstrated in the higher plants the existence of specific substances produced in special cells which stimulate growth in other cells, and so in the plant as a whole.

These so-called auxines are essentially hormones. Section B will soon be listening to an account of their chemical nature.

It is of particular importance to my present theme and a source of much satisfaction to know that our knowledge of the actual molecular structure of hormones and vitamins is growing fast. We have already exact knowledge of the kind in respect to not a few. We are indeed justified in believing that within a few years such knowledge will be extensive enough to allow a wide view of the correlation between molecular structure and physiological activity. Such correlation has long been sought in the case of drugs, and some generalisations have been demonstrated. It should be remembered, however, that until quite lately only the structure of the drug could be considered. With increasing knowledge of the tissue structures pharmacological actions will become much clearer.

I cannot refrain from mentioning here a set of relations connected especially with the phenomena of tissue growth which are of particular interest. It will be convenient to introduce some technical chemical considerations in describing them, though I think the relations may be clear without emphasis being placed on such details. The vitamin, which in current usage is labelled "A," is essential for the general growth of an animal. Recent research has provided much information as to its chemical nature. Its molecule is built up of units which possess what is known to chemists as the isoprene structure. These are condensed in a long carbon chain which is attached to a ring structure of a specific kind. Such a constitution relates it to other biological compounds, in particular to certain vegetable pigments, one of which  $\beta$ -carotene, so called, is the substance which I have mentioned as being convertible into the vitamin. For the display of an influence upon growth, however, the exact details of the vitamin's proper structure must be established. Now turning to vitamin D, of which the activity is more specialised, controlling as it does the growth of bone in particular, we have learnt that the unit elements in its structure are again isoprene radicals; but instead of forming a long chain as in vitamin A they are united into a system of condensed rings. Similar rings form the basal component of the molecules of sterols, substances which are normal constituents of nearly every living cell. It is one of these, inactive itself, which ultra-violet radiation converts into vitamin D. We know that as stated each of these vitamins stimulates growth in tissue cells. Next consider another case of growth stimulation, different because pathological in nature. As you are doubtless aware, it is well known that long contact with tar induces a cancerous growth of the skin. Very important researches have recently shown that particular constituents in the tar are alone concerned in producing this effect. It is being further demonstrated that the power to produce cancer is associated with a special type of molecular structure in these constituents. This structure, like that of the sterols, is one of condensed rings, the essential difference being that (in chemical language) the sterol rings are hydrogenated, whereas those in the

cancer-producing molecules are not. Hydrogenation indeed destroys the activity of the latter. Recall, however, the ovarian hormone oestrin. Now the molecular structure of oestrin has the essential ring structure of a sterol, but one of the constituent rings is not hydrogenated. In a sense therefore the chemical nature of oestrin links vitamin D with that of cancer-producing substances. Further, it is found that substances with pronounced cancer-producing powers may produce effects in the body like those of oestrin. It is difficult when faced with such relations not to wonder whether the metabolism of sterols, which when normal can produce a substance stimulating physiological growth, may in, very special circumstances be so perverted as to produce within living cells a substance stimulating pathological growth. Such a suggestion must, however, with present knowledge be very cautiously received. It is wholly without experimental proof. My chief purpose in this reference to this very interesting set of relations is to emphasise once more the significance of chemical structure in the field of biological events.

Only the end results of the profound influence which minute amounts of substances with adjusted structure exert upon living cells or tissues can be observed in the intact bodies of man or animals. It is doubtless because of the elaborate and sensitive organisation of chemical events in every tissue cell that the effects are proportionally so great.

It is an immediate task of biochemistry to explore the mechanism of such activities. It must learn to describe in objective chemical terms precisely how and where such molecules as those of hormones and vitamins intrude into the chemical events of metabolism. It is indeed now beginning this task which is by no means outside the scope of its methods. Efforts of this and of similar kind cannot fail to be associated with a steady increase in knowledge of the whole field of chemical organisation in living organisms, and to this increase we look forward with confidence. The promise is there. Present methods can still go far, but I am convinced that progress of the kind is about to gain great impetus from the application of those new methods of research which chemistry is inheriting from physics: X-ray analysis; the current studies of unimolecular surface films and of chemical reactions at surfaces; modern spectroscopy; the quantitative developments of photo-chemistry; no branch of inquiry stands to gain more from such advances in technique than does biochemistry at its present stage. Especially is this true in the case of the colloidal structure of living systems, of which in this address I have said so little.

#### IV

As an experimental science, biochemistry, like classical physiology, and much of experimental biology, has obtained, and must continue to obtain, many of its data from studying parts of the organism in isolation, but parts in which dynamic events continue. Though fortunately it has also methods of studying reactions as they occur in intact living cells, intact tissues, and,

of course, in the intact animal, it is still entitled to claim, that its studies of parts are consistently developing its grasp of the whole it desires to describe. However, even if that grasp may be from analysis. Justification for any such claim has been challenged in advance from a certain philosophic standpoint. Not thus that of General Smith, though in his powerful address on "The Mind and the Content of Meaning" he, like many philosophers to-day, emphasized the importance of properties which emerge from systems in their categories. I did not remember that a part whole in the whole is not the same as the part as a relation. He listened to about in a subsequent speech, but even, that for experimental biology, or for any other branch of science, it may be useful and necessary to approach the whole through its parts. Not even in the claim challenged to the standpoint of such a teacher as H. N. McCoy, the author in philosophy of organic mechanism there is no real crisis of analysis with its internal and multiple relations, and each whole is more than the sum of its parts. I nevertheless find no justification in his system which directly or indirectly the methods of bacteriology. In the teaching of J. M. Haldane, however, the value of such methods have been directly challenged. Some here will perhaps remember that in his address to Section I, twenty five years ago, he described a philosophic study, not which he has consistently maintained in many variations since. His Haldane told that to the enlightened biologist a living organism does not represent a problem for analyzing it as an organism, systematic. Its essential attributes are systematic; heredity, for example, is not high as not a problem for an animal. "The problem of physiology is not to obtain mechanical physics explanation of physiological processes" (I quote from the 1915 address), "but to discover by observation and experiment the relationships to one another of all the details of structure and activity in such organism as expression of its nature as one organism." I cannot pretend adequately to discuss these views here. They have often been discussed by others, not always perhaps with understanding. What is true in them is subtle, and I doubt if their author has ever found the right words in which to bring to most others a conviction of such truth. It is involved in a world outlook. What I think is scientifically faulty in Haldane's teaching is the *a priori* element which leads to bias in the face of evidence. The task he sets for the physiologist seems vague to most people, and he forgets that with good judgment a study of parts may lead to an intellectual synthesis of value. In 1885 he wrote: "That a meeting-point between biology and physical science may at some time be found there is no reason for doubting. But we may confidently predict that if that meeting-point is found, and one of the two sciences is swallowed up, that one will not be biology." He now claims indeed that biology has accomplished the heavy meal because physics has been compelled to deal no longer with Newtonian entities but, like the biologist, with organisms such as the atom proves to be. Is it not then enough for my present purpose to remark on the significance of the fact that not until certain atoms were

found spontaneously splitting piecemeal into parts, and others were afterwards so split in the laboratory, did we really know anything about the atom as a whole.

At this point, however, I will ask you not to suspect me of claiming that all the attributes of living systems or even the more obvious among them are necessarily based upon chemical organisation alone. I have already expressed my own belief that this organisation will account for one striking characteristic of every living cell—its ability, namely, to maintain a dynamic individuality in diverse environments. Living cells display other attributes even more characteristic of themselves; they grow, multiply, inherit qualities and transmit them. Although to distinguish levels of organisation in such systems may be to abstract from reality it is not illogical to believe that such attributes as these are based upon organisation at a level which is in some sense higher than the chemical level. The main necessity from the standpoint of biochemistry is then to decide whether nevertheless at its own level, which is certainly definable, the results of experimental studies are self-contained and consistent. This is assuredly true of the data which biochemistry is now acquiring. Never during its progress has chemical consistency shown itself to be disturbed by influences of any ultra-chemical kind.

Moreover, before we assume that there is a level of organisation at which chemical controlling agencies must necessarily cease to function, we should respect the intellectual parsimony taught by Occam and be sure of their limitations before we seek for super-chemical entities as organisers. There is no orderly succession of events which would seem less likely to be controlled by the mere chemical properties of a substance than the cell divisions and cell differentiation which intervene between the fertilised ovum and the finished embryo. Yet it would seem that a transmitted substance, a hormone in essence, may play an unmistakable part in that remarkable drama. It has for some years been known that, at an early stage of development, a group of cells forming the co-called "organiser" of Spemann induces the subsequent stages of differentiation in other cells. The latest researches seem to show that a cell-free extract of this "organiser" may function in its place. The substance concerned is, it would seem, not confined to the "organiser" itself, but is widely distributed outside, though not in, the embryo. It presents, nevertheless, a truly remarkable instance of chemical influence.

It would be out of place in such a discourse as this to attempt any discussion of the psycho-physical problem. However much we may learn about the material systems which, in their integrity, are associated with consciousness, the nature of that association may yet remain a problem. The interest of that problem is insistent and it must be often in our thoughts. Its existence, however, justifies no pre-judgments as to the value of any knowledge of a consistent sort which the material systems may yield to experiment.

## V

It has become clear, I think, that chemical modes of thought, whatever their limitations, are fated profoundly to affect biological thought. If, however, the biochemist should at any time be inclined to overrate the value of his contributions to biology, or to underrate the magnitude of problems outside his province, he will do well sometimes to leave the laboratory for the field, or to seek even in the museum a reminder of that infinity of adaptations of which life is capable. He will then not fail to work with a humble mind, however great his faith in the importance of the methods which are his own.

It is surely right, however, to claim that in passing from its earlier concern with dead biological products to its present concern with active processes within living organisms, biochemistry has become a true branch of progressive biology. It has opened up modes of thought about the physical basis of life which could scarcely be employed at all a generation ago. Such data and such modes of thought as it is now providing are pervasive, and must appear as aspects in all biological thought. Yet these aspects are, of course, only partial. Biology in all its aspects is showing rapid progress, and its bearing on human welfare is more and more evident.

Unfortunately, the nature of this new biological progress and its true significance is known to but a small section of the lay public. Few will doubt that popular interest in science is extending, but it is mainly confined to the more romantic aspects of modern astronomy and physics. That biological advances have made less impression is probably due to more than one circumstance, of which the chief, doubtless, is the neglect of biology in our educational system. The startling data of modern astronomy and physics, though of course only when presented in their most superficial aspects, find an easier approach to the uninformed mind than those of the new experimental biology can hope for. The primary concepts involved are paradoxically less familiar. Modern physical science, moreover, has been interpreted to the intelligent public by writers so brilliant that their books have had a great and stimulating influence.

Lord Russell once ventured on the statement that in passing from physics to biology one is conscious of a transition from the cosmic to the parochial, because from a cosmic point of view life is a very unimportant affair. Those who know that supposed parish well are convinced that it is rather a metropolis entitled to much more attention than it sometimes obtains from authors of guide-books to the universe. It may be small in extent, but is the seat of all the most significant events. In too many current publications, purporting to summarise scientific progress, biology is left out or receives but scant reference. Brilliant expositions of all that may be met in the region where modern science touches philosophy have directed thought straight from the implications of modern physics to the nature and structure of the human mind, and even to speculation concerning the mind

of the Deity. Yet there are aspects of biological truth already known which are certainly germane to such discussions, and probably necessary for their adequacy.

## VI

It is, however, because of its extreme importance to social progress that public ignorance of biology is especially to be regretted. Sir Henry Dale has remarked that "it is worth while to consider to-day whether the imposing achievements of physical science have not already, in the thought and interests of men at large, as well as in technical and industrial development, overshadowed in our educational and public policy those of biology to an extent which threatens a one-sided development of science itself and of the civilisation which we hope to see based on science." Sir Walter Fletcher, whose death during the past year has deprived the nation of an enlightened adviser, almost startled the public, I think, when he said in a national broadcast that "we can find safety and progress only in proportion as we bring into our methods of statecraft the guidance of biological truth." That statecraft, in its dignity, should be concerned with biological teaching, was a new idea to many listeners. A few years ago the Cambridge philosopher, Dr. C. D. Broad, who is much better acquainted with scientific data than are many philosophers, remarked upon the misfortune involved in the unequal development of science; the high degree of our control over inorganic nature combined with relative ignorance of biology and psychology. At the close of a discussion as to the possibility of continued mental progress in the world, he summed up by saying that the possibility depends on our getting an adequate knowledge and control of life and mind before the combination of ignorance on these subjects with knowledge of physics and chemistry wrecks the whole social system. He closed with the somewhat startling words: "Which of the runners in this very interesting race will win it is impossible to foretell. But physics and death have a long start over psychology and life!" No one surely will wish for, or expect, a slowing in the pace of the first, but the quickening up in the latter which the last few decades have seen is a matter for high satisfaction. But, to repeat, the need for recognising biological truth as a necessary guide to individual conduct and no less to statecraft and social policy still needs emphasis to-day. With frank acceptance of the truth that his own nature is congruent with all those aspects of nature at large which biology studies, combined with intelligent understanding of its teaching, man would escape from innumerable inhibitions due to past history and present ignorance, and equip himself for higher levels of endeavour and success.

Inadequate as at first sight it may seem when standing alone in support of so large a thesis, I must here be content to refer briefly to a single example of biological studies bearing upon human welfare. I will choose one which stands near to the general theme of my address. I mean the current studies of human and animal nutrition. You are well aware that during the last

twenty years—that is, since it adopted the method of controlled experiment—the study of nutrition has shown that the needs of the body are much more complex than was earlier thought, and in particular that substances consumed in almost infinitesimal amount may, each in its way, be as essential as those which form the bulk of any adequate dietary. This complexity in its demands will, after all, not surprise those who have in mind the complexity of events in the diverse living tissues of the body.

My earlier reference to vitamins, which had somewhat different bearings, was, I am sure, not necessary for a reminder of their nutritional importance. Owing to abundance of all kinds of advertisement vitamins are discussed in the drawing-room as well as in the dining-room, and also, though not so much, in the nursery, while at present perhaps not enough in the kitchen. Unfortunately, among the uninformed their importance in nutrition is not always viewed with discrimination. Some seem to think nowadays that if the vitamin supply is secured the rest of the dietary may be left to chance, while others suppose that they are things so good that we cannot have too much of them. Needless to say, neither assumption is true. With regard to the second, indeed, it is desirable, now that vitamin concentrates are on the market and much advertised, to remember that excess of a vitamin may be harmful. In the case of that labelled D at least we have definite evidence of this. Nevertheless the claim that every known vitamin has highly important nutritional functions is supported by evidence which continues to grow. It is probable, but perhaps not yet certain, that the human body requires all that are known.

The importance of detail is no less in evidence when the demands of the body for a right mineral supply are considered. A proper balance among the salts which are consumed in quantity is here of prime importance, but that certain elements which ordinary foods contain in minute amounts are indispensable in such amounts is becoming sure. To take but a single instance: the necessity of a trace of copper, which exercises somewhere in the body an indispensable catalytic influence on metabolism, is as essential in its way as much larger supplies of calcium, magnesium, potassium or iron. Those in close touch with experimental studies continually receive hints that factors still unknown contribute to normal nutrition, and those who deal with human dietaries from a scientific standpoint know that an ideal diet cannot yet be defined. This reference to nutritional studies is indeed mainly meant to assure you that the great attention they are receiving is fully justified. No one here, I think, will be impressed with the argument that because the human race has survived till now in complete ignorance of all such details, the knowledge being won must have academic interest alone. This line of argument is very old and never right.

One thing I am sure may be claimed for the growing enlightenment concerning human nutrition and the recent recognition of its study. It has already produced one line of evidence to show that nurture can assist nature to an extent not freely admitted a few years ago. That is a subject

which I wish I could pursue. I cannot myself doubt that various lines of evidence, all of which should be profoundly welcome, are pointing in the same direction.

Allow me just one final reference to another field of nutritional studies. Their great economic importance in animal husbandry calls for full recognition. Just now agricultural authorities are becoming acutely aware of the call for a better control of the diseases of animals. Together these involve an immense economic loss to the farmers, and therefore to the country. Although, doubtless, its influence should not be exaggerated, faulty nutrition plays no small share in accounting for the incidence of some among these diseases, as researches carried out at the Rowett Institute in Aberdeen and elsewhere are demonstrating. There is much more of such work to be done with great profit.

## VII

In every branch of science the activity of research has greatly increased during recent years. This all will have realised, but only those who are able to survey the situation closely can estimate the extent of that increase. It occurred to me at one time that an appraisal of research activities in this country, and especially the organisation of State-aided research, might fittingly form a part of my address. The desire to illustrate the progress of my own subject led me away from that project. I gave some time to a survey, however, and came to the conclusion, among others, that from eight to ten individuals in the world are now engaged upon scientific investigations for every one so engaged twenty years ago. It must be remembered, of course, that not only has research endowment greatly increased in America and Europe, but that Japan, China and even India have entered the field and are making contributions to science of real importance. It is sure that, whatever the consequences, the increase of scientific knowledge is at this time undergoing a positive acceleration.

Apropos, I find difficulty as to-day's occupant of this important scientific pulpit in avoiding some reference to impressive words spoken by my predecessor which are still echoed in thought, talk and print. In his wise and eloquent address at York Sir Alfred Ewing reminded us with serious emphasis that the command of Nature has been put into man's hand before he knows how to command himself. Of the dangers involved in that indictment he warned us; and we should remember that General Smuts also sounded the same note of warning in London.

Of science itself it is, of course, no indictment. It may be thought of rather as a warning signal to be placed on her road: "Dangerous Hill Ahead," perhaps, or "Turn Right"; not, however, "Go Slow," for that advice science cannot follow. The indictment is of mankind. Recognition of the truth it contains cannot be absent from the minds of those whose labours are daily increasing mankind's command of Nature; but it is due to them that the truth should be viewed in proper perspective. It is, after



but I think few men of affairs seriously believe what is yet probable, that the replacement we are thinking of will impose a new structure upon society. This may well differ in some essentials from any of those alternative social forms of which the very names now raise antagonisms. I confess that if civilisation escapes its other perils I should fear little the final reign of the machine. We should not altogether forget the difference in use which can be made of real and ample leisure compared with that possible for very brief leisure associated with fatigue; nor the difference between compulsory toil and spontaneous work. We have to picture, moreover, the reactions of a community which, save for a minority, has shown itself during recent years to be educable. I do not think it fanciful to believe that our highly efficient national broadcasting service, with the increased opportunities which the coming of short wavelength transmission may provide, might well take charge of the systematic education of adolescents after the personal influence of the schoolmaster has prepared them to profit by it. It would not be a technical education but an education for leisure. Listening to organised courses of instruction might at first be for the few; but ultimately might become habitual in the community which it would specially benefit.

In parenthesis allow me a brief further reference to "planning." The word is much to the front just now, chiefly in relation with current enterprises. But there may be planning for more fundamental developments; for future adjustment to social reconstructions. In such planning the trained scientific mind must play its part. Its vision of the future may be very limited, but in respect of material progress and its probable consequences, science (I include all branches of knowledge to which the name applies) has at least better data for prophecy than other forms of knowledge.

It was long ago written, "Wisdom and knowledge shall be stability of thy times." Though statesmen may have wisdom adequate for the immediate and urgent problems with which it is their fate to deal, there should yet be a reservoir of synthesised and clarified knowledge on which they can draw. The technique which brings governments in contact with scientific knowledge in particular, though greatly improved of late, is still imperfect. In any case, the politician is perforce concerned with the present rather than the future. I have recently read Bacon's *New Atlantis* afresh and have been thinking about his Solomon's House. We know that the rules for the functioning of that House were mistaken because the philosopher drew them up when in the mood of a Lord Chancellor; but in so far as the philosopher visualised therein an organisation of the best intellects bent on gathering knowledge for future practical services, his idea was a great one. When civilisation is in danger and society in transition might there not be a House recruited from the best intellects in the country with functions similar (*mutatis mutandis*) to those of Bacon's fancy? A House devoid of politics, concerned rather with synthesising existing knowledge, with a sustained appraisal of the progress of knowledge, and continuous concern with its bearing upon social readjustments. It is not to be pictured as composed

of scientific authorities alone. It would be rather an intellectual exchange where thought would go ahead of immediate problems. I believe, perhaps foolishly, that given time I might convince you that the functions of such a House, in such days as ours, might well be real. Here I must leave them to your fancy, well aware that in the minds of many I may by this bare assertion lose all reputation as a realist!

I will now hasten to my final words. Most of us have had a tendency in the past to fear the cult of leisure to the majority. To believe that it may be a great social benefit requires some mental adjustment, and a belief in the educability of the average man or woman.

But if the political aspirations of the nations should grow sane, and the artificial economic problems of the world be solved, the combined and assured gifts of health, plenty and leisure may prove to be the final justification of applied science. In a community advantaged by these, each individual will be free to develop his own innate powers, and, becoming more of an individual, will be less moved by those herd instincts which are always the major danger to the world.

You may feel that throughout this address I have dwelt exclusively on the material benefits of science to the neglect of its cultural value. I would like to correct this in a single closing sentence. I believe that for those who cultivate it in a right and humble spirit, science is one of the humanities; no less.

## THE SPIRIT OF MODERN BIOCHEMISTRY

[Orvosi Hetilap, 1935, 79, 1046]

CHEMISTRY when concerned with the phenomena of life finds for itself two separate main lines of endeavour. While the final goal of both lines is the same their approach to the living organism is different, and each requires its own special equipment for success. The first is the endeavour to determine the constitution and properties of all the materials found in association with life, and the second, which to-day seems the more ambitious, is to follow so far as may be possible the dynamic chemical events in which those materials play their parts while still within a living system. From the days of Liebig until the end of the last century the first endeavour was diligently pursued by a few. It continues still in more numerous hands and though progressing rapidly is by no means complete. The second line of endeavour had made relatively little progress before the present century began. Indeed most biologists had felt that its pursuit was outside the scope of chemical science. It was their faith that the subtle molecular events which must underlie the visible functions displayed by living organisms are initiated and controlled by the inherent but elusive potency of protoplasm in its integrity. They felt therefore, with apparent reason, that the methods of the chemist, which must destroy that integrity, could not illuminate this field of biological reality.

Early in the present century however a forward step was made which awakened new courage in biochemical endeavour. Its importance was at first not recognised by all, but it was fated to lead to a real change of outlook. I refer to the realisation of the fact that the chemical reactions which underlie and support the manifestations of life do not necessarily cease on the destruction of the living complex. Their progress is due to a phenomenon very familiar in inorganic nature. They are catalysed reactions, controlled by catalytic agencies, which, though part of the complex, retain therein a definite individuality. These biological catalysts are the intracellular *enzymes*. Enzymes as catalysts with extracellular functions had of course been known long before, the digestive enzymes in particular; but only some thirty years ago did it become clearly recognised that others, acting within the living cell, have much wider and still more significant functions.

With this knowledge the biochemist no longer stands powerless in the presence of a vital complex supposed to be insusceptible of profitable analysis. As a justifiable first step towards an understanding of that complex, he is busily engaged in analysing it into a number of separate but still active systems, each comprising a specific enzyme isolated from the complex, together with those particular constituents of the living system which suffer change under its influence. Intensive studies of such isolated

systems are continually proceeding, leading to a knowledge of the nature of the intermediate and final products of change and to an understanding of the influence of conditions on the progress and velocity of each reaction.

I have engaged in such studies myself and for some years have had the good fortune of seeing them carried out by many colleagues in the laboratory under my charge. As one who tried, but failed, to think profitably about the dynamic chemistry of living systems before the functions of intracellular enzymes were realised, I can bear witness to the enlightenment that the realisation has provided.

Experimental science, however, sorely needs a warning from philosophy that a whole is something more than the sum of its parts! Having learned all he can about those individual biochemical events which still progress when isolated from a living system, the biochemist yet realises that his ultimate aim is, within the capacity of his methods and from his own particular angle, to throw light on the nature and behaviour of living organisms when intact. While the isolated events he studies are biological and real - for the part can contain no factor that does not pre-exist in the whole - it is certain that in that high organisation of internal events which is the outstanding characteristic of every organism no one chemical reaction proceeds in complete independence of others.

Now it is easy to study the chemical balance sheet of an intact living organism, comparing the materials which it receives from its environment with the end products of change which it returns to that environment; but all the complex internal steps and stages of change, knowledge of which is so essential to a proper understanding of the chemical aspects of life, for the most part elude study in the intact animal. But we know now that the ambition to discover just how biochemical reactions are organised when they proceed in natural union in living tissues is no unreasonable one. To-day we are justified in attempting it. As a highly profitable further step towards that ambitious end the biochemist takes advantage of the circumstance that the life of individual organs and tissues when they are isolated may long survive that of the body from which they are taken.

The death of the individual not infrequently depends upon a purely local and maybe mechanical occurrence. The heart, say, from some local cause, is arrested and as a result all other tissues are fated to cease their activities. But this result, though rapid, is by no means instantaneous and only follows because the oxygen and other factors which the circulation brings to them, and the removal of waste products which it secures, are essential to their equilibrium. No subtle entity leaves the tissues at the moment of individual death! If we replace the blood-stream quickly enough by a suitable fluid artificially circulated, we can maintain the normal activity of the organs for quite long periods after they have been removed from the body. There are, indeed, some tissues that will thus "survive" without the application of any such technique. Such are the muscles of cold-blooded animals like the frog. These, as every junior student of physiology knows, retain

their normal power of contraction long after they have been removed from the animal, especially if they are placed in pure oxygen instead of air. More than thirty years ago, the late Walter Fletcher and I were able to follow the course of important chemical events in these organs with a success which was great enough to encourage later investigators to go much further. Our knowledge of the chemical events in active muscle is now remarkably extensive.

But to preserve the activity of the organs of mammals after isolation we must, without delay, provide them with an artificial circulation, or adopt some other plan for securing an oxygen supply for the still living cells. I will not stop to discuss the methods which have been used for securing the former, though they have yielded results of value.

But there is a much simpler method of maintaining and studying the processes of survival life. To the uninitiated it may seem crude and too simple, but it has been astonishingly successful. Some years ago Otto Warburg found that in order that the living cells in any organ shall receive the various requirements for survival life, it is only necessary to cut the organ immediately it is removed from the body into slices so thin that oxygen can easily penetrate to each constituent cell. These slices are rapidly transferred into a suitable medium with a suitable atmosphere in contact with it. Modern methods of micro-analysis, it should be mentioned, permit the use of very small weights of tissue. It is encouraging and even astonishing to realise with what success highly significant knowledge is now being obtained at many centres by the use of this simple but effective technique. We are learning by its use the precise nature of many chemical reactions which are proper to the cells of this or that organ. We can follow the sequence and relations in which they occur, and we can follow the course of important processes from start to finish. I have been in touch with the progress of biochemistry for half a century and am convinced that no single step has done so much as the adoption of this simple technique to bring it nearer to its ultimate, if still distant, goal. It is progressing from the study of substances to that of events. The study of organisation lies before it.

There is however one branch of inquiry, important to biochemistry no less than to physiology, in which the intact animal must still be employed. I mean the study of nutrition in its more general aspects.

Most are aware that research during recent years has made it sure that the nutritional needs of the body are more complex than was earlier believed. Among its more subtle demands of course is that for a proper supply of vitamins—those food constituents which although actually consumed in such minute amounts are yet so essential. I have been described sometimes as the “discoverer” of vitamins; but to receive that title gives me some discomfort of mind. As in so many other cases of advance in knowledge the existence of vitamins constituted no clean cut discovery by an individual. When I first entered this field it was not uncultivated. I can only claim that my early work made it clear that the importance of dietetic factors

functioning in minute amount was true in the broad domain of physiology and not in the narrower one of pathology alone. I was perhaps the first to challenge an earlier orthodoxy in nutritional science.

No one who reads the current literature of vitamin research, which of late has comprised as many as a thousand publications in a single year, can fail to be impressed with the increasing complexity of the facts revealed. While we have definite knowledge of various essential vitamins, those who take part in the experimental side of nutritional research cannot fail to realise that there are factors in nutrition still unknown to-day. They may not all belong to the category of vitamins narrowly defined. The time has come indeed when the data which have accumulated from animal experiments are calling for critical survey and discriminative evaluation. Nevertheless it is sure that the reality and wide importance of vitamin functions are becoming increasingly evident.

While so much scientific complexity is being revealed the practical application of this newer knowledge of nutrition involves relatively simple considerations: for well balanced diets of natural foods so far as can be seen cover all needs. We have now knowledge which seems to be adequate for the definition of such diets. The only difficulties in the way of its application for the benefit of the world's populations are economic. It is to be recognised that certain foods which have too often been luxuries for the rich are really necessities for all.

There remains however a point of some interest. Individual vitamins are now available in highly concentrated preparations, or in a pure form. What is the therapeutic value of these? In conditions clearly recognised as deficiency diseases their use is obvious; but have they wider applications? Clinicians have done well to reserve judgment on this, but careful reading of a large literature brings a conviction to-day that nutritional deficiencies play a larger part in the causation of disease, or in the failure to resist disease, than has hitherto been admitted, and vitamin-therapy may well have an important future before it.

In any case a truly great advance in knowledge has arisen from the isolation of vitamins in a pure state and the determination of their actual constitution.

I cannot refrain from mentioning in illustration of this advance the isolation of the anti-scorbutic vitamin, which we now know as ascorbic acid, by Professor Albert Szent-Györgyi of Szeged. I feel pleasure in remembering that his brilliant researches which led to that accomplishment began in the School of Biochemistry at Cambridge, though as the century has done fine independent and personal enterprise of one whose genius has done fine service to other branches of biochemistry.

In conclusion, let me urge that in emphasis on objective lines the highly specific and potent influence of such agencies as hormones and vitamins is to-day an immediate task for biological science. The biochemist cannot fail to believe that each one of these in its own peculiar way must influence

those dynamic chemical processes which he is now studying. It must be his future task to describe in clear chemical terms just how these influences are exerted. A generation ago such a task would have seemed impossible, but modern biochemistry can to-day attempt this, and similar tasks, in a spirit of confidence and optimism.

# THE NATURALIST IN THE LABORATORY

[*London Naturalist*, 1936, p. 49]

I would like first to acknowledge the high honour the London Natural History Society has done me by choosing me as its Honorary President. Doubtless owing to my own fault, this is the first opportunity I have had of expressing verbally the gratitude I feel for the honour thus done me.

In following so distinguished a predecessor as Lord Grey of Fallodon I cannot fail to realise the dignity of the office, and I am proud to know that the Society should think me worthy to hold it. I would like to say further that I appreciate the catholicity displayed in the Society's choice of a laboratory worker for that office, and especially perhaps of a *liter* chemist, whose activities might seem remote from the interests of a Natural History Society.

Most of your members in their approach to the problems of Science have, I imagine, followed paths very different from those which fate of late years has allotted to me. Many, and I suppose the majority, devoted themselves to the study of plants and animals when these are only themselves; when they are displaying in freedom the characters which express their innate qualities, independent of and untroubled by their higher environment. There, I feel, may well seem to be the true standpoint.

Many others among you are concerned with the direct study of nature with the meaning and history of the human body, of those in which life is displayed. For this understanding and the education of the colleges are essential and the resources of the museum and the services of their staff specialists are indispensable. We cannot leave the task of education to those who follow the path of investigation. They must cooperate in their laboratory.

[illegible]

fluid media. Is he not then too far removed from Nature to be a Naturalist? I hope you do not think so, for I covet the title!

I remember many years ago watching side by side with an accomplished lepidopterist the alternate darting and hovering of a Hummingbird Hawk Moth, and I remarked, "What marvellous little engines those wing muscles must be. How one would like to know how they work." "And do you hope," said my friend, who worshipped the beauty of the display (and to this I may claim I was by no means indifferent), "do you hope to learn this with pestle and mortar and test-tube?" I understood his feelings, but I am presently going to claim that the biochemist, during the years that have elapsed, has made some progress at least towards an understanding of how those highly efficient motors burn their fuel.

I take pride in the biochemist's progress in such directions, yet in speaking to a Natural History Society I almost feel that I should apologise for the destructive habits of his kind. But if my cursory and little prepared remarks can be said to have a purpose, it is to ask you to admit that all of us who find an abiding interest in the multitudinous manifestations of life are naturalists under our skins. We must all bear our specialised labels, but under that pleasing old designation we may all find a place.

I would like straightway to express my convictions that biological science for its progress has yet need of service from competent workers of all kinds. I do not sympathise with the view which is sometimes met to-day that descriptive work in biology has had its day and that the experimentalist is now the only progressive worker. The field observer, the skilled collector, and the taxonomist are still continuously helping us to advance and have much yet to do. To this point I will return. In the history of biology the study of form and outward behaviour inevitably came first; that of function came later and is even now less advanced. The study of function soon calls for the method of controlled experiment, and all should rejoice, I think, to know that there are twenty experimental biologists to-day for each one that was at work half a century ago.

Speaking as a laboratory worker to a Natural History Society, I am glad to remember that the highly-talented biologist whom that Society is honouring this evening, though from his earliest days a lover of Nature in the wild, became a highly-skilled inhabitant of the laboratory without losing any of his earlier love.

During those years when, with extraordinary skill, Bacot was illuminating our understanding of how the very small may enable the infinitely small to bring death to the big, and helping to show how that calamitous ability may be circumvented, he never ceased being a true naturalist.

It is sometimes of interest to try and discover how far something innate in each one of us has helped—circumstances apart—to determine the paths we have followed.

I will here venture, hoping for your forbearance, to intrude a fragment of personal history into my remarks. Like Bacot himself and, I suspect,

like very many of my audience, I was in my early days an ardent collector of butterflies and moths and (next easiest, I think, for the boy or the amateur) of beetles. It was one day in March of the year 1878—it is just 57 years ago—that I first made, with infinite pleasure, the acquaintance of the little bombardier beetle, *Brachinus crepitans*, if that be still its accepted scientific designation. It was plentiful that year and I found it in a northern suburb of London and on the South Downs near Eastbourne. Now, when one day I beheld, without previous knowledge of their abilities, how these insects on being disturbed eject a violet vapour into the air, a most effective act for offence or defence, I felt an intense curiosity to know not only how this volatile stuff could be made and stored but in particular what the stuff could be. I tried a few experiments, putting one bombardier after another into the same test-tube, and encouraging each one to shoot. The vapour condensed on the side of the covered tube and I thus collected a little of the material. It was very little, however, and I was a youth with no chemical training, so nothing came of my researches. I think, however, that from that time my fate was sealed. Though the designation was not yet invented, I became there and then a biochemist at heart.

I am reminded by a friend of a circumstance that I had quite forgotten, namely, that in the volume of the *Entomologist* for 1878 is published a note of mine on the capture of these beetles, with remarks upon the frequency of their occurrence in the Spring of that year. This was my first scientific publication. I find it contained no account of my experiments, but this I think was due to the shyness of youth.

I have learnt from Professor Major Greenwood's admirable account of his life and work that Bacot's first contribution to natural history appeared in 1893, fifteen years after mine, but much more important. It interested me to know that Bacot's paper dealt with pigments of a lepidopterous insect, those of the larva of *Saturnia corporis*. For I myself, a few years after I had made the acquaintance of *Brachinus* and his ammunition, became interested in the pigments of lepidoptera. In the early eighties I noticed that the wing pigments of coloured *Pieris* were soluble in water and felt that this gave one a chance of discovering their nature. To make a long story short, I became early convinced that these pigments were derivatives of uric acid, while the wings of white *Pieris* contained uric acid itself. I had thus come across a case of the use of excretory substances in ornament, a phenomenon which may shock or please the aesthetic sense according to the point of view. My dealings with these pigments, owing to the need of a multitude of insects and to lack of leisure, were not published in full till 1895, the date of Arthur Bacot's first long contribution to science. The latter dealt widely, you may remember with the genus *Carduelis*, but a subsidiary part of the paper was concerned with the purpurine coloration in the larva of *S. ocellatus*.

I am venturing to speak thus of my own early scientific adventures in connexion with the awakening of Bacot's genius as an experimentalist,

because I think the outcome may perhaps illustrate the truth of my earlier suggestion that something innate in each one of us may, apart from or even in spite of circumstance, determine the scientific paths (or other paths) that we are to tread. Bacot, like myself, seems to have started with an early interest in pigments, but the innate tendency of his mind led him to think straightway of their direct biological interest, in relation for instance to protective coloration, just as the same tendency carried him, through genetical studies and the like, to his classical work in medical entomology. I, on the other hand, though I would like to think that I am a biologist at heart, could not escape the curiosities of the chemist or refrain from his destructive pursuits.

I will here, by the way, just mention in parenthesis that my work on the pigments of the Pieridae has suffered, forty years after it was published, a correction which had I been younger might have seemed to me a tragedy, but now I can bear it with equanimity. These pigments, yellow and red, are sure enough uric acid derivatives, but the parent material in the scales of the white species is not, as I thought, uric acid itself but a related substance with very similar properties not hitherto known to chemists. The distinguished organic chemist Heinrich Wieland, who has made this correction, admits that for long he himself believed the substance to be uric acid, and it was, *inter alia*, the fortunate application of a discriminate qualitative test, non-existent in my time, which first led him to the distinction, so that I do not suffer greatly in pride over this happening. There is, after all, some uric acid in these white wings, though the newly described related substance is in largest amount. The biological significance of the facts remains. One among the facts has always interested me. These excretory pigments are universal in the Pieridae, but apparently sharply confined to them. The colours of other groups, in so far as they depend on pigmentation and not on physical effects, are due to quite different substances. Even when a Pierid successfully mimics an individual of another family, each insect, in spite of the similarity of colour schemes, employs pigments which chemically are typical of its own group. This confirms the soundness of a classification based on morphological considerations alone, but offers also an illustration of an aspect of affairs that I will emphasise later, namely, that a *chemistry* of species always underlies or is associated with the *morphology* of species. But I have said more than I intended on this early work of my own.

Let me here repeat the hope that every field naturalist and systematist in my audience agrees with me in thinking it fortunate that experimental biologists are to-day increasing in numbers. The experimental zoologist, the plant physiologist, the experimental geneticist, and others of their kind, are supplying knowledge concerning life of a kind which descriptive biology is powerless to supply, knowledge of which there was very little before the present century began.

The study of organic functions in the higher animals—what may be called classical physiology—has been long the hand-maid of Medicine. This,

of course, must remain its most important practical function. But as a branch of disinterested biological science physiology was, in consequence of its medical commitments, too long preoccupied with the mammal or, at any rate, with the vertebrate, alone. Comparative physiology, however, is now a progressive science, and is broadening our understanding of the relations between function and form. This might be illustrated from recent work done on various phyla, but on this occasion at least, with Bacot in mind, we are justified in thinking particularly of insects. For the study of form as adjusted to function (or vice versa) and the effect of environmental influences upon both, there is surely no group more interesting than this multitudinous one. In its evolution Nature has herself been a great experimentalist. In her apparent desire to make representatives of the group ubiquitous and able to cope with most diverse environments, she has had to overcome many difficulties in spite of a remarkable series of morphological adaptations.

I am sure that many here must have read the admirable little monograph on insect physiology by my friend (and, if on small grounds I may so call him, my old pupil) Dr. Wigglesworth, of this school. One has been apt to think that relatively little work had been done in this field, yet the author confesses to having consulted 2,000 references in the course of writing his book. Only a small proportion of these I imagine can have been equal in interest to the publications of Wigglesworth himself and by others in Professor Buxton's department, but the figure illustrates the extreme activity of scientific research during the last few years. It is sure that insect physiology is a fertile field from which the harvest has as yet been very incompletely garnered. Nevertheless, the survey in question is full of fascinating facts largely unknown to the average physiologist, who is primarily concerned with the mammal.

As the exploration of function progresses *chemical* problems are bound to arise, and then the biochemist may claim the right to intrude. Modern biochemistry took origin from classical physiology and on its own lines it, too, is concerned with function. Its formal or academic separation from the parent discipline—a separation which to-day is becoming common—is only justified by the fact that biochemistry needs wider contacts than does "physiology" as academically defined, and more, I think, than the study of the visible functioning of organs, it calls for wide variety in its materials. Without the inclusion of micro-organisms, for instance, as well as the tissues of green plants and animals, a full understanding of the essential nature of the physical basis of life could not be reached.

You will not, I think, feel resentment if I here attempt a very brief appraisal of the present position of biochemical science. The Society has taken some risks in inviting a specialist who is also an enthusiast to address it.

I myself remember the days when many, if not most, biologists looked askance at the chemist who ventured to pretend that his methods might help to throw any significant light on life's activities. It was his business,

they felt, to deal with the dead and not with the living. It is my desire that you should believe that modern biochemistry has attained to no inconsiderable success in illuminating many aspects of these activities; to success which justifies faith in its future.

The first and more orthodox task of the chemist as an organic chemist is doubtless to determine the nature and molecular structure of biological substances, but the chemist as a biochemist must not stop there. It is for him to strive for an understanding of the dynamic events in which these substances, or some of them, take part when the tissues are alive and functioning.

The pursuit of what we might call the morphological or static side of biochemistry began long ago, and is exceptionally progressive to-day. Perhaps some of the most interesting aspects of its progress are of the following kind. First, the realisation that though biological substances are multitudinous there are fundamental types of molecular structure which seem to be peculiarly adjusted to subserve the activities of the living. I am not thinking so much of the materials obtained from the environment, but rather of those which actually support the essential structures of the living cell or which display active functions within it. In addition to the all-important proteins there are other substances of which the molecular structure seems to be of almost universal importance. For brevity's sake I will mention only two classes of such, sterols and pyrrol derivatives. For my purpose here I need not stop to recall the actual chemical formulae of these substances. The fundamental structure in each case is a complex association of carbon rings, nitrogen being present in the pyrrols but not in the sterols.

Now it is a familiar laboratory experience that even a comparatively slight modification in the architecture of a molecule, which does not affect its essential type, may involve subtle and even unexpected changes in its individual properties, although the more general properties of its type may be retained. In the differentiation of biological function in its chemical aspects we seem to find economy in the employment of molecular types, advantage being taken of the effect of adjustments within the type for the production of substances exercising various necessary functions. Thus, in the case of substances of the sterol type, while one at least plays an important part in determining the static properties of the surfaces of living cells, in another the sterol structure is so modified as to give it dynamic influence as a sex hormone, while yet another among the sterols functions as a vitamin. The pyrrol structure in certain associations confers that quality upon chlorophyll which enables it to trap solar energy. In other associations it confers upon the respiratory pigments which contain it the power of carrying molecular oxygen from the air to the tissues, while modified, and in different associations, the same structure functions in promoting the actual oxidations which occur within the tissues; quite a different function from that of oxygen transport.

Such facts, while illustrating the chemical parsimony of Nature, also illustrate a second aspect of *chemical morphology* which is of great importance in her general scheme, one to which I have already referred. I mean the chemical differentiation in species which underlies the differentiation in their outward forms.

It is now a good many years since immunological studies proved to us that though all proteins belong, as it were, to one chemical genus, those which characterise each species are themselves specific. The protein type is maintained but the molecular pattern of the tissue proteins varies from species to species. But such specific differences are not confined to the proteins. Comparative biochemistry continues to reveal differences in chemical make up which may characterise different phyla, different genera, or may be narrowed to differences between species. No less than the morphologist, the biochemist can provide evidence for relationship as well as for differences, and evidence even for probable lines of descent.

Such claims require illustration for their ready acceptance, but time compels me to pass on to another claim on behalf of biochemistry which I am especially concerned to make. I mean its success along the path which I have already suggested it is fated to follow, the path which should lead to an understanding not merely of the nature of dead biological materials but of the dynamic molecular events which are characteristic of life. It was this which not so long ago was held by many to be a presumptuous aim and an impossible task. I would like, however, to convince those who have not had the opportunity of appraising it that real progress has been made towards achieving that aim.

The first step towards it was the gradual understanding of how, at biological temperatures and amid the general conditions found in living cells, the chemical reactions which support life can occur at all. The fact that they do occur was long sufficiently mysterious to be one of the chief supports of vitalism. The vague view that the occurrence of these reactions resulted from the instability of protoplasm—an instability supposedly increased by entry of oxygen into the protoplasmic complex—was as little helpful to effective thought about them as was the assumption of a vital force.

The mystery disappeared with the realisation that every living system is the seat of catalytic influences similar to those familiar in the inorganic world, but displayed with an efficiency and an ordered complexity which are characteristic of living systems alone. Most here will, I think, have a general idea of the meaning of the term catalysis, though, to say the truth, although it is one of the most fundamental of phenomena, easily recognised and observed, its precise mechanism is in its details still a matter for speculation. It may be thus thought about: we may picture that in a given chemical system molecules present are potentially able to undergo change in particular directions, and are potentially capable of reacting with other molecules. But at temperatures such as those which are biological the fields of force

which hold the atoms together within them are so distributed and equilibrated that the molecules remain stable and inert. Such equilibrium may be disturbed by a rise of temperature in the system and activity induced, but not commonly at temperatures compatible with life. What has made possible the chemical happenings which are so essential to life as we know it is the circumstance that at biological temperatures the same activity may be conferred upon molecules by catalysis, a phenomenon which occurs when they merely make momentary contact with surface structures of a particular kind. The deep significance of this remarkable phenomenon (for though familiar it is yet remarkable) in the manifestations of life should be obvious. Such is the nature of these manifestations that we cannot imagine their display save in association with the colloid state of matter. But colloidal systems are unstable above certain critical temperatures which are low compared with those necessary for the progress of most chemical reactions. All colloidal systems, on the other hand, are characteristically the seat of surface phenomena. In addition to their external surface they display a relatively great area of internal surfaces; in the cell, the surfaces, for instance, which separate colloidal particles from the more fluid part of the cytoplasm. It is at such surfaces that catalysis may take place. What is necessary for its occurrence is, first, that the molecule to be activated should find at the surface a structure or pattern so related to its own structure that a temporary union follows when contact is made; secondly, that the nature of the union should be such as to modify the distributions of forces (or, in other words, the electron orbits) in the molecule so that it becomes active; and, lastly, that the union should be a temporary one which rapidly breaks down with the activated molecule directed into some definite path of change and the catalyst restored to its original condition, so that it can continue its influence upon an indefinite number of similar molecules. Just such catalytic surfaces are possessed by the intracellular enzymes of which every living system contains so many. The enzymes, though special in their kind, are in the nature of their action essentially similar to the catalysts employed by the chemist in his laboratory and on a vast scale in chemical industries.

To understand why active chemical events occur in each living tissue at biological temperatures we, therefore, no longer need to look to some mysterious property of protoplasm. They are determined by objective agencies which we can study individually. It is not sufficient, however, to realise how chemical reactions are initiated in cells and tissues. We have to picture them as controlled and organised. It is characteristic of every living unit and, of course, a necessity for the maintenance of its integrity and individuality that all chemical events within it should be highly organised. All the reactions must be regulated in respect of their velocity, sequence and mutual relations, so that each proceeds as part of an organic whole. To determine how this organisation is maintained is a more difficult problem than to decide why individual reactions occur.

There is, however, an aspect of biological catalysis which, in my belief, contributes significantly to this organisation. It is highly specific. Each intracellular enzyme is able to activate one kind of molecule alone or, at most, only closely related molecules. A given reaction, therefore, can only occur at all when the right enzyme is present in the right place. Its velocity will depend upon the amount of enzyme surface available for its catalysis and its direction is determined by the precise nature of the enzyme. There are circumstances which make for organisation. This is a theme I would like to develop, but to do so here and now is not possible. My aim has been to recall to your minds the deep significance of the phenomenon of catalysis in the living world. Life is manifested in chemical systems which it seems must have remained inert but for its influence. One would dearly like to know whether life at its origin was displayed by systems which could dispense with it, and, if not, what was the nature of the primitive catalysts.

The recognition of intracellular enzymes and their functions enabled the biochemist to begin his experimental analysis of the events in a living cell. He has learnt how to separate individual enzymes, to place each in contact with the substance it activates, and so to follow *in vitro* the nature and course of individual reactions. Another advance in technique has enabled him to follow the reactions in any particular tissue as they occur in due sequence and relations. Of late years we have learnt that tissues, after removal from the body, if given proper conditions, can long survive with their normal activities essentially intact. With the technique of tissue culture, survival and growth may, as you know, be maintained indefinitely. But we can make preparations of almost any animal tissue such that in proper apparatus we can follow quantitatively the progress of chemical events within it—those involved in its respiration, for instance—and by the use of the micro-methods of analysis now so developed we can follow the exact course of reactions in small amounts of tissue with noteworthy success.

Such studies are relatively new, but it may be claimed, I think, that very significant knowledge has been gained by such methods. You will recall the scorn with which my lepidopterist friend of long ago viewed any claim that chemistry could possibly follow the events in active muscle. Yet we have now illuminating knowledge of the complicated chemical events associated with the contraction of muscle. We know that a whole series of chemical reactions, mutually related and interlocked, intervene between the oxidation which yields the energy for contraction and the conversion of that energy into the mechanical work done by the muscle. We seem, moreover, to be on the verge of knowing just why and how the contraction occurs when this energy is made available. To take but one other example of success: we have learnt much about the nature and course of the oxidations which occur in a great variety of animal tissues. We know a good deal concerning the enzymes concerned; those which activate the molecules to be oxidised, and other agents which activate the oxygen or bring it into effective relations with the former. Knowledge of this kind is rapidly

accumulating and some of it is of a kind which was never even pictured as possible of attainment by physiologists thirty years ago.

Before leaving this laudation of my own subject, let me refer in fewest possible words to another aspect of the progress of biochemistry, or let us say of chemical physiology, if the distinction is worth while. This will, I suspect, be more familiar to you than the more recondite aspects I have been discussing: I mean the proof of the extraordinarily wide influence of hormones, so called, in the organisation of the animal body. The realisation of this has surely justified the application of chemical methods of thought to biology. For the highly specific influence of each hormone, differing so widely from that of each of the others, can only be due to its specific molecular structure, and final knowledge will only come when we know just why that particular structure exerts its particular influence upon this or that tissue. It is remarkable indeed to know how the properties of particular molecules dominate the field of physiology. The work of Otto Loewi, recently extended by the brilliant researches of Sir Henry Dale and his colleagues, has taught us that even the control of activities by the nervous system does not follow directly upon the arrival of a physical impulse travelling along the nerves. These investigations have proved that a specific substance is first liberated at the nerve ending and the observed effects are due to the influence of this upon events in tissues or organs. From a local effect like this we pass to the widespread influences of circulating hormones in great variety. Their function is dynamic; in a sense at least they are catalytic. And I would not have you separate too widely in your thought the vitamins of food from the hormones made in the body. Fundamentally, their functions are very similar. In surpassingly small amounts both can profoundly influence dynamic events. It is, I think, an accident of evolution, so to speak, that the body can produce some such agencies at home while others it must import.

I could in any case hardly have refrained from touching on the subject of hormones, but I was further tempted to do so for a particular reason. It is so interesting to get confirmation of the belief that, in spite of that diversity of chemical characters among species on which I have insisted, there are fundamental relations common to them all.

I return to insects! More than one observer seems to have obtained evidence that hormones may function in the physiology of some insects at least. I wish, however, to refer particularly to some recent work of Dr. Wigglesworth dealing with the control of moulting and metamorphosis in the hemipterous *Rhodnius prolixus* by a hormone secreted in the head and perhaps by a special glandular organ, the so-called corpus allatum. This work is outstanding for the ingenuity of the experiments employed and the convincing nature of the evidence obtained. The stimulus for the production of this hormone is dilatation of the abdomen. At a critical period after a meal, but not before, the blood can be shown to contain the hormone, since such blood will induce moulting in individuals which live long but

never otherwise moult owing to the removal of their heads. Introduced at the right periods this hormone-containing blood will produce precocious metamorphosis at early nymph stages. The same agency induces both moulting and the final stage of metamorphosis, and it would seem that it is another inhibitory hormone produced in very small amount which during the nymph stages prevents the premature occurrence of the latter. The story is a fascinating one which, had he lived to hear it, would certainly have given deep pleasure to Bacot.

Familiarity should not diminish our interest in this remarkable device of nature, this specialisation among a group of tangible chemical substances (many of which the chemist can already make in the laboratory and keep in bottles on a shelf) which serve to initiate, control and organise physiological events, nearly always produced in minute amounts yet with such properties that each by its intrusion into some mechanism is able to produce results which are so impressive and so fundamental.

The revelation of the existence of the properties and wide influences of intracellular enzymes and of hormones and vitamins has certainly justified the application of chemical methods and chemical thought to the phenomena of life. All of them are parts of most fascinating chemical mechanisms.

I quite believe that there may be some here who find it rather hard to sympathise with this concern about mechanisms, especially among those to whom I referred in my opening remarks; those of you who love to study living beings when they are truly themselves, when they are engaged upon their proper pursuits and triumphing often so marvellously over the difficulties which the world presents even for the humblest of its inhabitants. It may, indeed, be that gifted minds with adequate intuition may after all when so engaged reach to a more real understanding of life and its mysteries than do those who pretend to go deeper by studying its physical basis.

But some seem born with an urge to see the inside of things; they suffer from a development and sublimation of the childish desire to see how the wheels go round. To judge from the facts which indulgence in this analytical habit has so far revealed, the chemical mechanisms which are associated with life form a closed system, self-sufficient and suffering no interference from influences which are above mechanism. This is an important conclusion, but I would like to suggest that in accepting it one is not committed to a philosophy which is mechanistic in its fundamental outlook.

If there be truth in the philosophic dictum that the properties of the whole are always something more than the sum of the properties of its parts, it would seem to be especially applicable to the living organism, and I think most of us feel intuitively that life must be something more than an association of mechanisms. Whether science by itself can reach a decision on such a matter or tell us anything about that "something more" only the future can tell. Such great matters, however, are out of place in so unambitious an address as this of mine is meant to be.

I will return, in concluding, to what I earlier claimed to be the main purpose of my remarks, namely, to urge that biological science for its further progress has still need of service from competent workers of all kinds, and that all such belong to the brotherhood of naturalists.

Let us admit that some few adjustments in policy may further assist that progress. It is, I think, reasonable to admit, for instance, that in the teaching of general biology in schools and at universities the experimental side should replace some at least of the dominant descriptive side, but not so as to lead to neglect of the latter. It would seem well, too, that our natural history museums, in so far at least as they are meant to educate the elementary student or the general public, should continue in the endeavour, costly in space and expenditure though it be, to illustrate the natural environment of species as well as the species themselves. So significant are the relations between the organism and its environment that the field worker, and even the collector, needs to bear them in mind. In parenthesis, I might remark that in a somewhat different sense the archæological members of the Society—to whom, I fear, my discourse can have made little appeal—well know how important it is to note and record the environment of the objects of their interest. The profound interest of the facts revealed by modern plant ecology illustrates the importance of biological associations, and every naturalist should in some degree at least be an ecologist. This means that the worker in the field who wishes to advance scientific knowledge should cultivate wide interests and, to-day, needs perhaps, like most others, special training for his work. On the other hand, I am sure that all students who are being trained in biological laboratories should as a matter of routine receive occasional instruction in the field. All these claims for the wider aspects of biology are justified, but I wish once more to protest against any suggestion that descriptive work is effete. The wise and discriminating collector and the systematist or taxonomist whom he serves have still abundant work before them of a kind which is still of fundamental importance.

Finally, let me again ask you to agree that all true biologists deserve the coveted name of Naturalist. The touchstone of the naturalist is his abiding interest in living nature in all its aspects.



ON THE HILLS NEAR THE CANADIAN BORDER AT THE TIME OF THE HARVARD  
TERCENTENARY CONFERENCE, 1936.

"All true biologists deserve the coveted name of Naturalist. The touchstone of the naturalist is his abiding interest in living Nature in all its aspects. . . ." (p. 280).



## THE INFLUENCE OF CHEMICAL THOUGHT ON BIOLOGY

Lecture at the Harvard Tercentenary Conference on Arts and Sciences

[*Science*, 1936, 84, 258]

THE latter half of the last century, though a period of such rapid progress alike in physical and biological science, saw inadequate contact between the thought of the chemist and that of the biologist.

It is true, and a familiar circumstance to those with an interest in the history of science, that when that half century began, organic chemistry and what we now term biochemistry were both yet in embryo, and were hardly to be distinguished. Justus von Liebig fathered them both.

It was the genius of Liebig that started modern organic chemistry on a triumphant career, and Liebig's great desire, one which directed his own efforts, was to see chemistry render full service to animal physiology and to agriculture. This ambition, in satisfactory measure, was not fulfilled during Liebig's own lifetime, and it is, I think, of some historical interest to decide why during years when scientific minds were so alert, so promising a field was cultivated by so few. At first I think certain personal attributes in leaders of thought contributed to the separation of chemistry from biology. Liebig himself, for instance, though so brilliant a chemist, lacked a biologist's instincts. When with great enthusiasm he came to apply his chemical knowledge to the living plant or animal his thought often went obviously astray and much of his theoretical teaching was instinctively and rightly rejected in biological thought. What was really so valuable in that teaching lost therefore some of its influence. Strange as it may seem the influence of another dominant mind of the time, that of Pasteur, did not altogether favour an approach between chemist and biologist. If Liebig remained too much the chemist, Pasteur, once he entered with such immense profit to science the biological field, became almost too much a biologist, at least in so far as he favoured the current belief that the activities of a living organism could be understood only by thinking in terms of that organism as a whole. Any analysis of the totality he held to be of little avail.

Although such influences played a part, the chief factor which delayed an approach to biology from the chemical side was doubtless the extreme vigour of the young science of organic chemistry itself. The rapid development of the magical synthetic art of the chemist provided him with substances made not by nature but by himself, the properties of which were in the highest degree suitable for the development of clear ideas concerning molecular structure. There was therefore for a long time only occasional temptation to go to the plant or animal for new material.

Meanwhile, the times were not ripe for a serious approach from the side of biology. Zoology and botany were still essentially observational and descriptive sciences, while chemistry was of course, from the first, experimental, a circumstance which in itself helped in their divorce. In the history of biology it was of course inevitable that the study of form should precede the study of function, and not surprising that concern with the chemical differentiation which might be associated with morphological differentiation, and the molecular events which must underlie all displays of active function, should come still later.

Moreover, at the very time when Liebig was still engaged in urging the claims of chemistry on biological thought, the long and intelligent study of plant and animal forms in nature at large reached its great triumph in establishing the truths of evolution which orientated the thought of humanity. It is not surprising that this new outlook and the many suggestions it gave for yet closer study of morphological differentiation and adaptation left the general biologist preoccupied with the manifestations of form for many years longer. On the other hand, vertebrate physiology, starting as the hand-maid of medicine, was from the first, and long remained, the most experimental of biological sciences; necessarily experimental because it is concerned with the study of function. Before the end of the last century it had, as you know, accumulated an impressive body of enlightened knowledge concerning the visible functioning of organs. It was in the service of classical physiology that modern biochemistry had its more immediate origin. In its studies of metabolism physiology necessarily entered the chemical field, and though for a long time such studies were somewhat superficial, largely because adequate chemical knowledge was lacking, they prepared the way for the more ambitious efforts of current biochemistry.

It is true that in Germany the chemical side of physiology was studied for its own sake, and in continuity right from the days of Liebig onwards. This was the case in the university of German Strasbourg. Here alone for many years was the subject of physiological chemistry recognised as entitled to recognition as a self-standing scientific discipline, and especially under the influence of the genius and the highly trained mind of Felix Hoppe-Seyler much fine work was done at this centre even during the years of which I have been speaking. Until the turn of the century, however, the progress of biochemistry was relatively slow and for the most part consisted in a gradual increase in knowledge concerning the general nature and distribution of the many chemical substances which are to be found in animal and plant tissues, together with continued enterprise in studies of the metabolic balance sheet of the human body which also led to much, but rather detached, knowledge of the renal secretions. Little became known of the actual chemical events which occur in the tissues during life.

Apart from that divorce between chemical and biological thought, of which I have already made much, there was a tendency in the latter which further discouraged attempts to probe the secrets of living cells by chemical

methods. Most biologists were content to ascribe the internal events of metabolism to the elusive properties of an entity impenetrable of profitable analysis; to the influence of protoplasm. There was, as I well remember, a widespread feeling that chemical studies which interfere with the full integrity of protoplasm can at most have chemical interest and must remain without bearing on the realities of biology. Looking back I find it interesting to recall that it was just when the last century gave way to this, that certain advances occurring together within the space of a year or two greatly helped to change a point of view which for the chemist was wholly inhibitory. I would instance the publication of Emil Fischer's brilliant work on the chemistry of proteins, the discovery of hormones, and in particular the recognition, too long delayed, of the fact that the progress of chemical events in the living cell is controlled by definite objective agencies, the enzymic catalysts. These and other aspects of new knowledge revealed at a critical moment started biochemistry in its more modern phase on a period of rapid progress.

I propose here to put before you in fewest possible words an appraisal of the present position and outlook of this branch of science together with a personal confession of faith in the significance of the knowledge which it is harvesting.

From the first, modern biochemical inquiry has had ambitions beyond that of determining the nature of the materials with which the processes of life make play, essential as such knowledge is for its progress. Its claim to be an independent branch of inquiry is and must be based on success in describing the molecular events which underlie the manifestations of life wherever they occur. It is dealing, and must deal, with the living and not with the dead. Success in such endeavour is recent, but is not to be thought of as altogether new. During many years a few individual workers dealing with evidence yielded by the intact organism doubtless brought to light facts bearing significantly upon the nature of chemical events as they occur within the living tissue. But such workers have till recently been rare and the facts won were too isolated to form a significant body of knowledge. The last twenty-five years, however, have seen not advances in technique alone but an extraordinary growth of interest in the chemical dynamics of living tissues. Recruits to their study have become very numerous and publications concerning them almost overwhelming. There is now much knowledge of a consistent and significant kind, as well as a harvest of facts ready to fall into place as knowledge further advances.

You will not expect in a brief address any discussion of technicalities either of methods or results. I will deal with certain aspects of the newer knowledge on the broadest lines possible. How many and how diverse are the chemical reactions which support even some of the simplest displays of function—the contraction of a muscle for instance—is becoming daily more evident. Numerous individual reactions on the other hand are being isolated from living systems and maintained in progress for successful study

*in vitro*. They are found to progress because controlled by specific catalysts—the intracellular enzymes, and knowledge concerning the nature of these active agencies though far from complete is rapidly growing. In not a few instances it has proved possible to follow *in vitro* reactions still proceeding in that ordered sequence which (as we have now a right to assume) reproduces their actual relations in a living tissue itself. In particular has success been met in the study of reductions and oxidations in living cells, all-important among chemical reactions as providing energy at the right time and in the right place for the physiological functioning of each cell or organ.

Important to productive thought about such matters is the growing confidence that the structure and configuration of molecules, which organic chemistry determines with such accuracy, has as great a share in deciding the origin, the influence and the fate of substances in living systems as it has in the laboratory. This was formerly disbelieved or ignored by those who were content to ascribe all chemical events in a living cell to the influence of protoplasm as an entity real in its activities only when intact. The biochemical outlook could not fail to be widened and chemical thought concerning living organisms stimulated when physiological studies first revealed the existence of hormones and the general nature of their functions. In this field, in the specific activities of hormones and of vitamins, which can be justifiably spoken of as exogenous hormones, we have outstanding examples of the dominant influence of molecular structure, which in the case of any of these agencies is already known. We find among them diversity of structure associated with diversity of action and are learning that just as in the laboratory so in living systems there is first the influence of molecular type, and then the added influence of special atomic groupings. Also as in the laboratory certain modifications in the molecule may have relatively little influence upon its physiological activity, others may profoundly modify it. It is well to recognise how dominant is this influence of molecular pattern—the special concern of the chemist—throughout the realm of life.

Biochemical studies owing to their early origin from the medical field have long dealt chiefly with the mammal. If, however, the science is to arrive at significant generalisations, or to decide, for example, what, in a chemical sense, is essential to the manifestations of life, and what is less essential though found in multitudinous variations of a central theme, it must extend its studies into fields as wide as possible. Fortunately, associated with growing activities in the field of general physiology, there is to-day an increasing interest in comparative biochemistry and much of fundamental importance is being learnt from this. Comparative studies have led to an acute realisation of the fact that biochemistry can render important help towards an understanding of development, evolution and heredity. Chemical differentiation underlies, or is associated with, morphological and functional differentiation, and to learn exactly what is the nature of such association is a fascinating task ahead. Of great promise for future studies is the recent proof first that definite chemical substances are concerned in specifically

evolving stages in the morphological differentiation of the growing embryo, and second that recognizable chemical factors are present in the genetic constitution of germ or sperm and are concerned with the carriage of the hereditary characters which appear in the developed organism. Further, we find in passing from one group of organisms to another, that a given chemical function may be served by different though related substances. There is a chemistry of species. To all such conclusions the study of the plant is contributing no less than that of the animal. Much aid to biochemical thought, moreover, is coming from the studies of micro-organisms, which deal with them not from the standpoint of pathology, but from that of general biology, regarding them as living systems with chemical activities and potentialities which partake of the marvellous. The field of biochemical investigation has now become very wide. It would seem that chemical thought must now accompany biological thought wherever it is employed.

So rapid a review as that I have put before you provides but a poor measure of the amount of progress made, but for those who were not previously familiar with the aims and activities of a relatively new branch of science it will perhaps suffice as an indication of their nature.

I would like to remark here that partly perhaps because of the special interest involved in the constitution of substances so remarkable in their functions as *hormones* and *vitamins* many eminent organic chemists with their special intellectual equipment have of late been attracted into the biological field, and other aspects of biochemistry are receiving increasing attention from physical chemists. No branch of science, moreover, will benefit more than biology from those newer methods of study which chemistry is just now receiving from physics. All this is of happy augury. So many problems await the united efforts of the physiologist and the pure chemist together with the biochemist acting as a desirable, and, as I believe, a necessary intermediary.

To the branch of science of which the aims and claims have been before you the contributions of American workers have been exceptionally great. All those to whom, like myself, it has been the concern of a lifetime owe a deep debt to the country.

At a time when, save in Germany alone, the subject was receiving scant attention in Europe, where academic recognition of its needs was almost absent, and workers very few, many able investigators were already engaged upon its problems here. Posts were provided for them at not a few centres carrying titles which implied that the subject was worthy of regard as an independent scientific discipline. This is a circumstance familiar to those who have been directly concerned with biochemistry and it is made patent to all readers of Dr. Russell H. Chittenden's admirable book, *The Development of Physiological Chemistry in the United States*.\* In that book it is also made clear how and by whom the seeds were sown from which grew ultimately

\* American Chemical Society Monograph, No. 54; pub. Chemical Catalog Co., New York, 1930.

that wide and generous interest in the subject of which I have spoken. They were sown by Dr. Chittenden himself. So far back as 1874 he was placed in charge of the first definitive laboratory of physiological chemistry in America, which had its place in the Sheffield Scientific School at Yale University. This was but a small beginning and at that time Germany alone could provide the knowledge and experience necessary for the teacher, no less than the taught. The year 1878 therefore found Chittenden at Heidelberg, working under Willy Kühne, in whose laboratory knowledge and experience were present in full measure. It is interesting to learn from Dr. Chittenden that his choice of Heidelberg was intuitive. It was an intuition which fully justified itself. Chittenden, with the experience gained, and with Kühne's inspiration added to his own innate gifts, returned to a Chair of Physiological Chemistry at Yale, a post which he occupied for forty productive years.

Much good work was done during the last two decades of the century, but, as Chittenden himself points out, the study of the subject was limited in outlook till near the end of that period, because it was taught almost exclusively from the standpoint of medical needs. Medicine has been the foster parent of the biological sciences and can thus claim their loyalty, but medicine has sometimes kept them too long in leading strings, not always realising their capacity for independent growth. It was mainly with dead materials and the composition of secretions and excretions that physiological chemistry subserving the then limited demands of medicine, was concerned. Only a few—in America for instance Newell Martin and Chittenden—foresaw that it should widen its activities, and only such as Graham Lusk and Lafayette B. Mendel who later helped it into wider fields. I have earlier claimed that it was just at the turn of the centuries that biochemistry entered upon a new period of growth and undertook more dynamic studies. This is well illustrated by the circumstance that in America a number of brilliant investigators entered the field in the opening years of the present century.

I would very much like to pay a tribute to some of these. So many are worthy of mention, however, that it is difficult to avoid the invidious task of selection. Perhaps you may let me mention a few to whose work and writings I am myself especially indebted, confining my references rather strictly to those whose work was in progress before the end of the first decade of the century. These were pioneers in the newer phase of biochemical progress. I will begin by mention of Christian A. Herter, whose work began and, alas, also ended during the period with which I am dealing. Directly and indirectly he served biochemistry well; directly by his own work, indirectly in many ways, but especially in two. *The Journal of Biological Chemistry*, which he founded and financed, has for more than thirty years continued to publish the results of researches, very many of which are among the most important ever carried out in the subject. But Herter also fostered the genius of Henry Drysdale Dakin, and for that our debt to him is great.

Dakin during the period of which I speak brought to the field of biochemistry great technical skill inspired by real chemical vision and an instinctive grasp of the nature of biological problems. He has never ceased to serve the subject nobly. I will next mention one who was a senior among these pioneers. His classical work upon the endocrine secretions began before the century, but it continued during those first ten years. He has done much admirable work since and has guided the thought and work of many younger men into profitable channels. To John J. Abel biochemistry owes much. The work of Otto Folin at Harvard stands out especially because of the help it gave then and afterwards to the efforts of others. In the fertile production of new methods of analysis Folin was supreme. Apart from this, however, our knowledge of metabolism would have had serious gaps had his own fine work been lacking. Lawrence J. Henderson's thought and work were in full activity during the period of which I am thinking, and just at its close appeared a classical publication on the equilibrium between bases and acids in the animal body which led the thought of every biochemist on to new and productive lines. What he did then and all that he has done and taught since called for that rare and philosophical quality of mind which he possesses in full measure. Thomas Burr Osborne's invaluable work on the chemistry of proteins begun before, but continued through these years, can never be forgotten. His extraordinarily profitable partnership with Lafayette B. Mendel, to which we owe so much of our knowledge concerning the biological value of proteins, began just after the decade closed, but I may be allowed to pay a tribute of admiration to the enterprise. The work of Henry L. Wheeler and Treat B. Johnson at Yale and the later work of the latter alone on the pyrimidine bases involved constitutional studies of the highest value to biochemistry; and equally valuable was that of Walter Jones, at Johns Hopkins University, on nucleic acid and related subjects. The work of P. A. Levene during those early years was also devoted to nucleic acids and was most valuable. The brilliant and innumerable constitutional studies he has carried out in later years represent great accomplishment. Then from the Hull Physiological Laboratories A. P. Matthews was publishing his earlier researches dealing with physico-chemical problems of much interest.

Apart from the work of those recognised officially as biochemists the subject in those days was benefitting greatly, if indirectly, by the calorimetric studies of Francis Benedict and Graham Lusk. To personal friendship with the latter and to the stimulating influence of his thought I myself owe more than I can tell. From his great book on nutrition I have derived many a lecture to students; I fear without due acknowledgment.

In the years which have followed on this pioneer period biochemistry in America has received increasing recognition, and has achieved remarkable successes. Among the very many who have contributed to this recognition and high accomplishment the nature of this occasion allows me the pleasure of mentioning just one. I have remarked already that recently the professed

biochemist has fortunately gained the interest and the help of eminent organic and physical chemists which, except for the great service of Emil Fischer, it for a long time lacked. Among these is one who is equally eminent in both these branches of chemistry; I mean the distinguished President of Harvard University. Dr. Conant's work on chlorophyll and on blood pigments is of outstanding importance, and no less important are his enlightened studies of reduction and oxidation potentials which bear with the utmost significance on urgent biological problems. No one who takes pleasure in the growth of the subject can fail in gratitude to the President.

The activities which have been so notable in this country are now proceeding to varying extents in every land where science is cultivated at all, and the attention given to biochemical problems is everywhere still increasing. Seldom, I think, in the history of science has any branch of inquiry enjoyed so great an impetus.

What, you may ask, from the standpoint of pure knowledge is the goal of these activities, and what will be their ultimate accomplishment? I have faith that in the end they will reach to a full description of living systems in so far as they are chemical systems. From a knowledge of individual events they will proceed to an understanding of the organisation of these events, that organisation which makes the organism. I can see no obstacle to the attainment of such an intellectual synthesis. When it comes it will involve a full understanding of many of life's manifestations; which is of course not to say that it will define life itself. If, however, the claim of biochemistry is to describe life, at any level, in chemical terms, biochemistry may come more under the eye of philosophy than perhaps any other branch of biology. There are schools of philosophy which will continue to ignore facts of a kind accessible to the chemist as being without significance in their search for reality, but there are other schools which must at least take note of them. In any case, there are biologists with philosophical leanings who still suspect that biochemical facts are of chemical interest only.

The chemist from his own standpoint hopes rather to gain understanding of whole organisms through a study of their parts.

But these are days in which there is much insistence on the fact that in the world-scheme only wholes can partake of reality. The truth, felt vaguely, but almost instinctively even by commonplace and uninstructed minds, that the whole is something different from the arithmetical sum of its parts, has been sublimated and raised to the status of a philosophical doctrine.

It is impossible at Harvard to forget the teaching of that profound philosopher Alfred North Whitehead, who came one day from Cambridge to Cambridge. We have his assurance that the conception of organism must replace in thought the unrelated or accidentally related entities which were the abstract units of Newtonian physics. Reality always involves relations internal and external, while an event and no static entity must be the unit of things real.

Biology from its very nature has never been much tempted to abstraction

and for it the organism has always been the only significant unit, while the living organism as it exists in time is essentially a directed event. The question that arises is whether the modern biochemist, in analysing the organism into the parts which he is best able to study, has so departed from reality that his studies have no longer biological meaning. I myself would venture to answer that question, if it troubles the minds of any, by saying that *so long as his analysis involves the isolation of events, and not merely of substances, he is not in danger of such departure.* We should learn little about the nature of an organism by being shown a collection of every substance it contains in stoppered bottles. Each isolated event on the other hand partakes at least of the nature of the whole organism. Even if on occasion it is but a single biochemical reaction it remains an event controlled and directed. True it has lost the influence of the environment provided by the whole organism and its progress may be modified in detail; but in detail only, not in its essential nature.

I do not find that Professor Whitehead doubts the validity of such an approach to the biological whole through its chemical parts. In his Lowell Lectures, published in his great book, *Science and the Modern World*, which claims that historically in its concern with organism physiology "put mind back into nature," he remarked that "viewing the question (of organism) as a matter of chemistry, there is no need to construe the actions of each molecule in a living body by its exclusive particular reference to the pattern of the complete living organism." He admitted that each molecule may be so affected by the pattern of the whole living system as to be otherwise than what it would have been if placed elsewhere, but suggested that "it would be entirely in consonance with the empirically observed action of environment if the direct effect of aspects as between the whole body and its parts were negligible." It is true, of course, that no molecule which is actually playing a part in dynamic events within an organism is the same as when it contributes to the contents of a bottle on the shelf of the chemist. It is different because it is activated, and may be undergoing transition, and, doubtless, the precise state at a given moment of every molecule in a living cell is determined by the state of the whole cell at that moment. Such relations, however, though so complex, are not of a kind which need escape the ultimate power of experiment to define.

It is sure, I think, that biochemical facts and biochemical thought will provide fresh aspects for biological thought. They will no less strengthen the object of biological science to serve humanity.

It is sure that if he can add to what the eye itself reveals an adequate mental picture of the invisible molecular events which underlie the visible, the biologist will gain increased understanding of the behaviour of every living thing. The physiologist, too, will add to his understanding of every living function; and the clinician, no less than the pathologist, will acquire a deeper insight into the significance of every departure from the normal. This is my faith, and I hope it may be yours.

## THE PACE OF SCIENCE

[Birkbeck College Foundation Oration, 1936]

The opportunity that I am now enjoying for addressing this College and its friends is one which I very greatly appreciate. I owe to the College a measure of gratitude for the help I received from it at a critical moment in my career, and I take pleasure in finding an occasion, though after nearly fifty years, on which to acknowledge my debt. You will agree that half a century is a considerable interlude between the incurring of a debt and its acknowledgment!

Indirectly the invitation to address you has afforded me another pleasure. While by no means wholly ignorant of the history of this College, my knowledge concerning some of its stages was vague. In order to qualify me somewhat better for my appearance here, your Principal kindly provided me last year with a copy of Delisle Burns' very admirable history, and I have read it with enormous interest. It presents a record of which the College may indeed be proud. In particular, one cannot fail to admire the determination which the institution has always maintained, sometimes in the face of serious difficulties and opposition, to remain a centre where the fundamentals of knowledge are taught, and to refuse the status, which outside influences have from time to time tried to force upon it in the past, of a place where narrow technical—so-called "practical"—teaching was alone to be provided. This higher ideal inspired the spirit of the institution from its earliest days as the Mechanics' Institute and found its full justification when Birkbeck College emerged as a constituent of a now highly progressive University, a constituent with unique functions of which the importance is great and, in my opinion, certain to increase in the future. As a matter of fact, it may be fairly said that this College nursed the ideals of a university before the University of London itself assumed its proper functions, and it is delightful to know that the day is approaching when its domicile will be more suited to its many activities and to its dignity than is the case to-day.

At two separate periods in my own life I acquired a special interest in this College; first an interest in its quite early history, and, much later, an interest in the educational opportunities it provided. If you will forgive a personal reference, I will tell you that when I was a boy I saw much of an old man whose generation was twice removed from my own. He was a great-uncle of mine, who in his young days had been a friend and an admiring disciple of Dr. Birkbeck. We had much more converse than can be common between an old man and a boy, our sympathy being due, I think, to the fact that he himself was always somewhat of a rebel, and in me he discovered a rebel in embryo. He talked to me so often and so vividly of the foundation and of certain activities of the Mechanics' Institute that I became quite

familiar with them. I mention this because I learned from this old man how feverish and even fierce had been the enthusiasm of an enlightened few for the movement initiated by Dr. Birkbeck. Its intensity was doubtless fanned by the obscurantist opposition of the many who held that the movement was revolutionary, dangerous and even evil. That bitter opposition from the class which had reaped the chief benefits from the industrial revolution to the education of the class that suffered most from it, is one of those incidents in our social history which we would fain forget. I may add that I gained from this old uncle of mine so vivid a picture of Dr. Birkbeck's personality that it is hard for me to realise that the 160th anniversary of his birth has already passed.

As I said, it was many years after I listened to my old relation's talk about this institution that I myself sought its help. The resolution to pursue a career in medicine or science came to me relatively late in life. I became an external student of the London University, and, in order to prepare for an examination, which I think was the Intermediate B.Sc., it became necessary for me to attend some practical science classes. At the Birkbeck, and there alone, I found the opportunity I needed. This was half a century ago. I did not stay very long, because shortly after I joined I was able to enter the Medical School of Guy's Hospital, but I received here efficient instruction which made it easy to pass an examination that I had somewhat feared. My debt to the College is real, and it is extremely pleasant to be able to acknowledge it.

I remember with some misgiving that in addressing you I am following four eminent public men, experienced orators in spite of their disclaimers at the time, and highly qualified by the nature of their avocations to speak, as they did, on very broad themes of profound interest to every Faculty in the College. I am limited, I fear, by the narrower outlook of an academic specialist.

As a title for this address I chose last year: "The Pace of Science." I thought it well not to alter it this year, though I approach the subject perhaps on rather different lines. I do not propose to touch on any technicalities at all, not even vitamins.

That scientific knowledge continues to grow with astonishing rapidity is a circumstance very familiar to all, even to those whose appraisal of the nature and implications of that rapid progress may be vague. But, familiar as the theme may be, occasional reflection upon it is, I think, to be justified. We have to bear in mind not only the social repercussions of this vast and rapid growth of knowledge, but also the intellectual reactions it is calling forth.

The desire to direct your minds to the subject on this occasion arose, I think, from the effects of a personal experience. It was my privilege for five years to occupy the Chair of the Royal Society, and in the course of a hundred sittings I heard descriptions of many brilliant researches in almost every branch of science. During those years indeed I became acutely

aware of the previous limitations in my knowledge as a specialist, and greatly impressed—almost oppressed—with the multitudinous directions in which that special kind of knowledge which experimental science provides was growing; everywhere at a racing pace. Its progress is not confined to the major and more familiar branches of science, but continues in many less known borderlands here and there, and in many highly specialised lines of inquiry of which the very existence may well be unknown to the majority.

It is true, of course, that for many successive generations in the past men's minds have been impressed by the rapid growth of science in their own days, but what we have to realise to-day is the continued acceleration of that growth. The time span for a given amount of progress gets shorter and shorter, and in the investigation of nature the law of diminishing returns does not seem to hold and probably never will hold. It is easy to understand why now the pace of science is growing ever faster. In the last twenty-five years the number of investigators in pure and applied science has enormously increased in all countries. They are now becoming very numerous even in the East. Research itself is more adequately financed and, to some extent at least, better organised than ever before. If we can really believe that this positive acceleration is likely to continue (and, should civilisation survive, it is hard to see why it should not continue); if at the same time we estimate fairly the remarkable effects of new scientific concepts on our minds and of applied science on our civilisation during the last fifty years alone, can we imagine what its influence will have accomplished even, say, a century hence? It is tempting, but perhaps not very profitable to try.

I certainly will not ask you to visualise a future on the lines of, say, Aldous Huxley's *Brave New World*, or even on the lines of Mr. Wells' *Things to Come*, good prophet as Mr. Wells has sometimes proved to be. Indeed, I do not intend this evening to say much of the social repercussions of science. We have heard a great deal of these lately; of the dislocations produced by supposedly overhasty science on the one hand and of the frustration of science due to the ineptness of our present social system on the other hand. You may well be somewhat tired of these themes. I propose rather to speak first and chiefly on the intellectual reactions which the great pace of advancing science may involve, realising, however, my own limitations in making such an attempt.

Will the pace ultimately become such that the extreme degree of specialisation involved in its pursuit must result in an enormous accumulation of facts which no human mind will be able to correlate or synthesise into any significant whole? Will each investigator of nature—to use a current cliché—come to know "more and more about less and less" until his knowledge becomes so isolated from that of each of his fellows that his pursuit will prove self-inhibitory? Will each would-be investigator find, as Lord Russell has suggested he may possibly find, that life is not long enough for him so to store his mind as to reach the growing frontier where

his work should begin even in the case of the most specialised branch of inquiry? Or, finally, will the human race as a whole get tired of questioning nature, and civilisation continue without further curiosity concerning the secrets of nature, as did the civilisations of the East through the ages? In speculations such as these many have indulged. We should, I think, have a robust faith that science will never commit suicide or suffer defeat on any of the above lines. I believe that its growth will increase rather than diminish man's curiosity about the universe and, what is equally important, he will acquire more and more curiosity concerning every scientific aspect of his own complex self.

Any appraisal of the intellectual influence of progressive science, as distinct from the social influence of its applications, must involve a recognition of the fact that more than any other form of human endeavour it supplies ever fresh materials for the stimulation and exercise of active and consecutive thought. It must be recognised, too, that more than once in its history science has provided data which have compelled a complete reorientation in thought: the new data won for the mind in the days of Galileo, for instance, which broke down so many barriers in the way of intellectual progress, or—to make a long jump—in the days of Darwin and Lyell, which established once and for all the conception of an evolving instead of a static universe, or, in our own days, the wonderful harvest gathered by atomic physics, which more than anything else has been responsible for that real revolution in thought through which we have recently passed. These are outstanding instances, but they do not stand alone. One need not be very bold to suggest that science may be now preparing materials for wholly unexpected intellectual revolutions of equal importance and that perhaps at its present rate of progress some such may not be so far ahead. I sometimes feel that it may be again the turn of biology, as it was in the days of Darwin, to accomplish a real revolution in thought, not any more, perhaps, from studies of form but from studies of function illuminated by a knowledge of the molecular events which underlie every manifestation of life. Physics and chemistry are every day drawing nearer to biology, and psychology, with much to learn, is shaking hands with physiology. These are significant approaches, and those who are able to peep behind the scenes a little feel that a dramatic moment is not so far off.

We are justified in making a distinction between the receptive and the intuitive qualities of the mind and those more active intellectual faculties which are employed in consecutive thought and reasoning. The former are, in a sense at least, a static possession; the latter are dynamic and fully realised only when in action. It is convenient and perhaps allowable, though quite unorthodox, to speak of that power of the mind which often enables it to appraise situations, values and the general significance of things without recourse to consecutive thought, as intelligence, distinguishing intelligence in this sense from the intellect in action. If you will allow me that use of the word, I want to suggest that, while creative literature

and art call and have always called chiefly, though, of course, not exclusively, for high intelligence, effective dealing with the multifarious data of growing science exercises to a greater extent and in a fashion all its own the more active processes of the mind—what we often call hard thinking.

It is true that the highly active mind of the pure mathematician has dispensed with such data, and, no less, the equally busy minds of many philosophers. With regard to the former, however, if we are to believe that great mathematician, Henri Poincaré—though I believe all do not agree with him—intuition and imagination intrude so much into the course of mathematical accomplishment that one must place the pure mathematician nearer to the poets than to those busy with obtaining data from the external world. Nevertheless, is it not true that mathematical thought, when it comes in contact with the data of science, is often directed into paths of unsuspected interest? Applied mathematics has frequently reacted upon pure mathematics and fertilised it. With regard to philosophy, that of the Schoolmen is a familiar instance of much reasoning and logic applied to unreal or inadequate data, and of the many schools of philosophy which have based their conclusions on the results of introspection alone it may perhaps be said that, because they thus studied the mind in isolation, they have always been unprogressive. A little later I shall be bold enough to say a few words on the relation between modern philosophy and current science.

The appeal of literature and art is, of course, as great to-day as ever, and their benefits reach an ever-increasing number. Their influence, based on the values they reveal, is permanent because they satisfy cravings innate in mankind. But is it not true that, while the moods and modes of literature and art are infinite in their variety, the basic materials with which they deal remain always essentially the same? It is not in their nature to reveal anything fundamentally new about the human attributes which they portray. Love and hate, joy and pain, jealousy and ambition—these and their interplay, with their effect upon human conduct, are their materials. They may present the play of human passions in their nakedness or as emotions refined and sublimated, but at bottom their materials are the same to-day as when the hexameters of Homer were fresh in men's ears, or when the Mycenaean artists wrought their works of beauty. Each generation can profit, if it will, from all that has been finely said or depicted in the past, or enjoy finding it all said or depicted again in fresh modes adjusted to that generation's own predilections. Is it not, however, the language and the form alone that are new? I am not sure that those whose interests are in other faculties than that of science will agree with that statement. If it is not true, I hope that it is at least provocative! No one doubts, of course, that great literature and art in which the old materials are continually revived, re clothed and illuminated by genius are among the greatest of human accomplishments and offer the easiest path for the escape of the spirit from the trivialities of life and the boredom of its routine.

Is it not also true that, while the materials provided for thought by

science cannot, for the great majority at least, make any similar emotional appeal, its progress is almost daily adding something new for the exercise of the active side of the intellect; wholly new paths for thought to pursue? All the stories concerning emotional adventure may have been told, but stories of the kind science has in particular to tell, stories of pure intellectual adventure, are only beginning.

We should, perhaps, remember that some have felt in the past and not a few profess to feel to-day, that the kind of data science provides when compared with the world of values, are trivial and of no importance. There are those who even feel that thought can get nearer to reality by ignoring them. One thinks of such beliefs as those of Wordsworth, Tolstoi, Carlyle, Ruskin in some of his moods, or the beliefs of many more recent writers and of many philosophers, past and present. Some hold indeed that the intuitions of the artist may in their own way come nearer to a revelation of reality than is reached by any other method of approach open to the mind. There are some too who feel that, even if science has given with one hand, she has taken away with the other. I will quote from the writings of that great naturalist and philosopher, W. H. Hudson. John Galsworthy has quoted the passage—with much approval. In Hudson's book, *The Purple Land*, we read: "Ah, yes, we are all seeking after happiness in the wrong way. It was with us once, and ours, but we despised it, for it was only the old common happiness which Nature gives to all her children, and we went away from it in search of another grander kind of happiness, which some dreamer—Bacon or another—assured us we should find. We had only to conquer Nature, find out her secrets, make her our obedient slave, then the earth would be Eden, and every man Adam and every woman Eve. We are still marching bravely on, conquering Nature, but how weary and sad we are getting! The old joy in life and gaiety of heart have vanished. . . ." Here we have words of fine flavour and most of us have moods in which they seem to ring true. But can we really believe in that previous unalloyed happiness which existed before man started to conquer Nature? Nature unconquered was often very cruel to man.

When trying at this point to decide how best I could express to you what I myself feel about the intellectual gifts of science, I recalled a page from an interesting and unprejudiced book by a very able writer. From this I will quote as putting my case better than I could put it myself. The passage is from *The Limitations of Science*, by J. W. N. Sullivan, which may be known to many of you here. After claiming, rightly or wrongly, that the whole method of thought upon which the Newtonian outlook reposed has been abandoned, the author continues: "This fact need not give rise to melancholy reflections. Science is a wonderful exhibition of stages in the greatest of human adventures, the intellectual adventure." Then, after a brief reference to the lack of progress in man's dealings with his own emotions, he remarks: "But there has certainly been a real and advancing exploration of the resources of the mind. Man has advanced from one

level of abstraction to another. He thinks thoughts that were never thinkable before; he has broken through mental barriers that never before were broken. Whatever may be true of the physical universe, it is true that the mental universe is constantly expanding." Later the author adds: "For the ordinary student the chief charm of science is that it acquaints him not only with a new body of ideas but with new modes of thinking. He experiences a veritable growth of consciousness." That conception of a veritable growth of consciousness due to the increase of material for active conscious thought is just what I am trying to claim as a merit of our rapidly growing science.

Returning to my starting point, the growth is proceeding so fast that, as I have said, some see disasters ahead. Assuming that the dangers are in any way real, is there, by taking thought to-day, anything that can be done to prevent them? Certainly not by attempting to slow progress; science has its bit in its teeth, or, rather, it is getting rid of every kind of harness.

In the realms of pure thought the philosophers are kings, and the scientists have neither the desire nor the ability to dethrone them. Unless we include the pure mathematicians among them—and, as I have remarked, they stand nearer to the poets than to experimental investigators—the scientists are for the most part manual workers. This manual work takes a great deal of time, it is apt to employ the mind with the particular rather than with the general, it frankly often leaves too little leisure for thought, and—which may be worse—it leads thought into narrow channels.

In parenthesis may I say here that I was struck, when reading the Orations of my predecessors, to find Lord Tweedsmuir, then John Buchan, wondering whether the young men of to-day read Epictetus, and he implied that they certainly ought to do so. Heavens, I thought, did young men other than, say, those in for Greats at Oxford or perhaps those studying in the Philosophical Faculty at Birkbeck College, ever think of reading Epictetus? The suggestion of large leisure involved in Lord Tweedsmuir's inquiry is intriguing but gave me some discomfort of mind. I did not read Epictetus when I was young, and I see no chance of ever doing so now. One would like to believe that even science students to-day can find leisure to read a little philosophy, but I fear very few will get down to the reading of Epictetus. On the other hand, students of this College need not go far afield to learn just how much philosophy matters to science or science to philosophy. No one has thought more about their relations than Dr. Joad or has written more ably about them.

I have sometimes wondered whether the philosopher, from that high standpoint whence he surveys the whole field of knowledge, ought not to do more than appraise or criticise the intellectual value of advancing science. Should not it be within his ability to offer some advice as to the paths on which its progress should to-day be most encouraged, for there may be some advantageous choice of paths? From the mountain top he should see further ahead than those who work below. I am thinking here, of course,

of the guidance of science as an intellectual pursuit and not as the source of social amenities. If this guidance were possible, it would be a very great service for philosophy to render. The philosopher may feel, however, that it is not his business, and, after all, it is most unlikely, even if desirable, that science in flood could ever be canalised.

Throughout its history philosophy, relying on introspection, long tended to neglect the kind of data that science provides, and, on the other hand, from the standpoint of science, it is commonly urged that philosophy has never come to agreed conclusions. The two have tended to look askance at one another. A good many scientists have wondered, I think, whether if the Greek intellect at its zenith had not unhappily come to despise the experimental approach to Nature the subsequent development of both philosophy and science might not have been different. One feels that they might have been more mutually helpful. Just now indeed there seem to be signs of increasing mutual interest, surely a fortunate circumstance. Modern advances in physics have come near to metaphysics and seem to have carried some scientific minds beyond the frontier. As I have just said, the Head of your own Philosophical Faculty has written much in his own brilliant fashion concerning the philosophical implications of science. I note that he views with disapproval the metaphysical structures which certain modern scientists have erected, in which the universe becomes wholly an externalisation of the scientist's own mind. I am personally grateful to him for his critical dealing with such views concerning reality. I have felt in my bones that they are unsatisfactory without being qualified to discover just at what point in reasoning their falsity arises. It is clear that on such matters philosophy is entitled to deal faithfully with the speculative side of scientific thought. I myself welcome Dr. Joad's insistence on the fact that science is, after all, a new thing and that is why man is still inept in its use. Our present relative ineptitude may affect the intellectual use of scientific data more than it has affected their practical applications.

On the other hand, it must be remembered that scientific facts are among the most obstinate of facts, and philosophy can no longer ignore them. How far, however, does the acquisition of these facts help us to understand the nature of reality?

No one, I think, has more ably discussed the probable limitations inherent in the scientific approach to reality than has Dr. Joad. In a number of publications he has discussed them with great conviction on his own part. Many of you doubtless heard and others must have read Dr. Joad's Haldane Memorial Lecture which was published last year. I myself have read it many times as a mental discipline, and I commend it to you for that purpose. In essence it is a defence of free will from the attack of scientific determinism, and its conclusions should be faced by those who wish honestly to appraise the intellectual limitations of science. The mental discipline provided by that lecture, however, is not confined to the conclusions; it is involved in understanding the arguments by which those conclusions are reached.

One such is based on what the author denotes by the term "Contradiction." He admits that it is an argument of a purely logical order, and therefore likely to incur the distrust of any English audience. (You may perhaps have seen that an able Chinese has lately ascribed the whole of our success as a race and nation to our complete lack of logic! He has made an extraordinarily good case). This particular argument of Dr. Joad's against determinism has fascinated me greatly, especially as I can never be sure whether it has convinced me or not. I cannot attempt to trace its development now, but it is extremely interesting and its conclusion should awaken the curiosity of those who have neither heard nor read it, for the conclusion is that "If what determinism says is correct, the determinism which says it cannot be correct." A notable conclusion! But in any case Dr. Joad's final statement concerning this fundamental matter is clear, namely, that while science is competent to tell us something about everything it is not in a position to tell us everything about anything. He holds, moreover, as we learn from his books no less than from the Haldane Memorial Lecture, that avenues other than science must be conceded to be valid for the approach to reality, including those offered by aesthetic and ethical experiences. At the same time he is sure that our strong conviction that our wills are free cannot be countered by anything that science can say. Very many if not all scientific workers will accept these conclusions to-day without discouragement, but most will wish to insist that those other avenues may not by themselves yield anything complete and that the ultimate value of the scientific approach to reality has yet to be fully revealed. Philosophy, ethics, art and literature are old; science is new. The philosopher no less than the scientist may yet be "inept" (to use Dr. Joad's word) in the use of its data, and in the future scientific data may be dug from deeper depths.

Perhaps, however, the chief conclusion we should reach in this great matter is that the working scientist must cultivate honesty of mind in facing the issues. He may not feel called upon to believe in the indeterminism which atomic physics are supposed by some to have revealed, and he certainly acquires a firm faith in determinism while in the laboratory. While there at least he must either believe in a mechanistic universe or admit that his methods by themselves fail to reveal all that is real in the universe. There can be no honest intellectual compromise in this case. Many Victorian writers compromised in vain.

I have made much of the danger inherent in the increasing isolation of the specialised investigator due to his almost inevitable preoccupation with the mass of new knowledge which he must continuously endeavour to assimilate within his own domain. Yet frequent exchanges across the frontiers of knowledge are essential for the symmetrical growth of science. I do not know whether other specialists will agree with me in my belief that a certain form of help for us may be forthcoming now. An increasing number of very able writers are to-day producing books in which the most significant aspects of the progress in this or that branch of science are

assembled and appraised with accuracy and literary skill. I am, of course, not thinking of efforts meant merely to popularise science. Even such books as those I have in mind are perhaps at present mostly written for the intelligent lay public, but the best of them are of real value to the specialist. I venture to think that every encouragement should be given to this form of literature, which at the high standard of which I am thinking is something new and of an importance which will steadily increase. It can be produced at its best only by men with special gifts who are enabled to give all their time to it. They must escape the exacting demands of personal teaching and research. I fear that within the world of what we may call official science insufficient recognition is extended to those who render it this indirect service. Honour in that world is won, for the most part, by original research work. Up to a point this is, of course, justified. New acquisitions to scientific knowledge can come only through research, and, this apart, every teacher of specialised science should himself be an investigator or his teaching may become stereotyped and dull. The spirit of research should be active in every teaching department, and this College must take pride in knowing that distinguished researches are carried out in connexion with all its Faculties and not those of science alone. It is indeed both remarkable and admirable that so much post-graduate work should be carried out by students who have only their spare time to devote to it. I venture to say that if there be any man of wealth, who wants to know the best way in which to help science or learning, I am quite sure that if he were to provide proper and ample funds for the higher class of research in this College he could not possibly make a better choice of method.

There are however some gifted individuals who find in themselves an inborn sympathy with the nature and aims of science together with an urge for literary expression rather than for experiment. If such elect to devote their lives to wide surveys of scientific progress and the interpretation of its significance for their colleagues who are immersed in specialised researches, I for one think that science should honour them as being among those who really advance it. In these days of specialisation this particular form of specialisation is surely justified. Should any student here possess the ability and desire to render this rather than any other service to science, although I am not in a position to speak as to the magnitude of its financial reward, I am perfectly certain that he will be welcomed by a very great number of his fellow scientists. I am thinking, of course, of the development of a literature in which the essential and significant items of current progress are selected with highly educated judgment and presented with literary skill, while what is speculative is avoided and no special weight is given to the sensational. I hope that you will not think these remarks are trivial. My very real desire has been to suggest that literary efforts of this kind should be encouraged by us all; they are likely to be of great help in the future.

I have said nothing so far about the social repercussions of the rapid

growth of science and the effect of the inventions based upon it. Just one reflection I will allow myself, before closing, on the well-worn theme of "Machine versus Man."

Ever since the advent of the industrial revolution discussions of the tyranny of the machine have arisen at intervals to disturb the public mind, especially, of course, whenever unemployment has been greater than usual. You will have noticed doubtless that, in his brilliant address as President of the British Association, Sir Josiah Stamp—and there is no better authority on such matters—showed that to-day at least the total net effect of the replacement of human labour by machinery is much less than is usually assumed. The gross displacement is much lessened by the development of new industries based on scientific advances, and many out of the total unemployed are so at any moment for temporary reasons only, while another proportion is represented by the unemployable. Yet, if this be true to-day, it has not been so, of course, throughout the history of industrialism.

The industrial revolution which brought wealth and power to the middle classes was associated with a code of morality among those who were its leaders which appears the more remarkable the more one thinks about it. In mid-Victorian times the rectitude of this particular code was unquestioned by the majority. That able French observer, writer, and student of the English, André Maurois, has said of these Victorians: "The invention of the steam engine and industrial machinery, and the astounding development of English railways and mines had inspired in them a passionate belief in material progress. The new science of political economy had taught them that the relations between men are not moral relations or duties but are decreed by laws no less real and inevitable than the law of gravity or the movement of the stars. The law of supply and demand was the gospel of these men, and Manchester their Holy City." Yet the majority of these consisted of highly respected and self-respecting men, austere in matters of the flesh, carefully avoiding the profane which they detected in all but very limited forms of literature and art, and for one day in seven devoted to a religion the tenets of which directly contradicted the teachings of the ethical code which guided them in practical affairs. It was, above all, this contradiction which made their outlook so remarkable, so worthy of being recalled and studied. Their attitude towards those who worked for them was painfully illustrated by their opposition to the Factory Acts, and they were chief among those who opposed the educational policy of Dr. Birkbeck as something dangerous and even evil.

Professor Whitehead, following one of his subtle lines of thought not always to be followed easily by ordinary minds, traces the appearance of this strange nineteenth century morality to the gradual effects of earlier influences; even to the influence of Descartes, who so sharply separated the affairs of the body from those of the mind or soul. This separation, Dr. Whitehead holds, as it permeated thought, removed mundane affairs from the realm of values altogether. It also led to the Protestant recoil

from aesthetic experiences depending upon anything material, and to that separation, in many Protestant countries at least, of religion from the practical affairs of life. However this may be, it is certain that very few among the Victorian industrialists were directly influenced by Descartes or had even heard of him!

The influence of the code of these industrialists was, of course, not confined to mid-Victorian times. In America for a couple of generations after the civil war it was applied more crudely and brutally than perhaps ever in this country, and we are reminded that its spirit is still alive when we read the opinions of Mr. Henry Ford as expressed in his book published in 1930. "We now know," wrote Mr. Ford, "that anything which is economically right is also morally right. There can be no conflict between good economics and good morals." We seem to gather, however, from the book that the interests of the industrialist must set the standard of goodness for both.

The leaders of Victorian industry in this country set a fashion, and it seems worth while to reflect that if they, as a special group of human beings devoted in their own way to duty, had been influenced by a more consistent ethical code, if they had been less completely other-worldly in their Sabbatarian religion, while thinking more of human values in the midst of their daily enterprises, the progress of science and invention might from the beginning have benefited all classes instead of only a few.

It was but natural that these men should have been actuated by the profit-motive, but as professors of a merciful religion they might well have believed that an enterprise should not begin to count profits before the demands of each social duty have been paid for as well as its material costs. Had they visualised the machine as an agent which, while still accelerating profits, could yet soften the lot of labour, and had they realised that if insecurity for the worker is inherent in the industrial system, then unemployment insurance of some kind should be a logical necessity and a natural part of the system; then, though wealth might have accumulated somewhat more slowly, there would have been less suffering in the past and a better balanced society to-day. I have recalled those days to your minds merely as a remarkable illustration of what we may call the frustration of science due to the inconsistencies of human nature.

In this country at least there has been of late a real awakening of the social conscience, and in economic affairs the responsibility of the country as a whole for the well-being of its parts is receiving more and more recognition. If civilisation survives its present dangers, the social benefits of science are in the future less likely to meet frustration, and it is certain that it has many yet to provide. Though its rapid growth may present for itself difficulties of the kind we have been considering, we may have faith that it is yet capable of benefiting both the minds and the bodies of mankind in ways beyond our present dreams. The pace is great, and the pace is growing greater.

# BIOLOGICAL THOUGHT AND CHEMICAL THOUGHT

## A PLEA FOR UNIFICATION

[Linacre Lecture, 1938]

IN preparing this lecture I have yielded to a temptation excusably felt, I think, by one who sees near at hand the close of an active career. To compare the present with the past in the field of his own activities, and to advertise the progress he himself has seen, is, for such, a natural impulse where opportunity is offered. If, however, I now yield to that impulse it is not without an ulterior aim.

No one with adequate knowledge will deny that the subject of biochemistry during recent years has shown itself to be a fertile field, at least for those intent on gathering a harvest of facts. The recruitment of labourers to that field has lately grown, and is still growing so fast that the facts garnered seem already overwhelming. In the minds of some at least, the question still arises: What (practical applications apart) is likely to be the ultimate significance for biological science of these myriad chemical facts? It is part of my intention to attempt in a very limited and humble way to make some appraisal of this significance.

To this end then I may first be allowed to estimate in fewest possible words the essential nature, as it appears to me, of the progress which biochemistry has made since I first had concern with it just fifty years ago. I can, of course, do no more than illustrate that progress with a few examples, dealing with its kind rather than its extent. I ought perhaps to say at once that when throughout this lecture I refer to its progress, I shall mean the advances it has made as a branch of fundamental scientific inquiry. Its practical applications in the field of medicine and elsewhere are to-day not without general recognition. On these I will not dwell on this occasion.

### OUTLOOK OF THE BIOCHEMIST

In my own earliest days all who hoped to apply chemical methods and chemical thought to biological problems suffered certain noteworthy disadvantages. First they obtained little help and no great sympathy from the organic chemists of the day, whose interests had for the most part wandered away from the study of natural products; then they felt acutely the lack of an adequate knowledge of colloids, for colloid science had been completely neglected after the pioneer studies of Thomas Graham, then thirty years old, and, again, they had yet to develop adequate methods for the service of what they conceived to be their own special needs. All disadvantages of this kind have happily disappeared to-day.

In those earlier days, however, the would-be biochemist suffered an inhibition of a different kind which, to some at least, myself among the number, was real. We had to face the distrust of the guardians of a territory which we hoped to invade. I am thinking in particular of the attitude of many experimental biologists, whose work just then was already revealing knowledge of great significance. Let me say here that encouragement for the chemist was not lacking from leading physiologists of that day; many of these believed that their own subject called for development on its chemical side. It was indeed that belief in the mind of one of the most eminent among them, the late Sir Michael Foster, which gave to me the opportunities I have for so many years enjoyed in Cambridge. But the professional interests of the physiologists were naturally centred mainly on the functioning of organs, or on the metabolism of the animal body as a whole, while the particular hope of the ambitious biochemist, when his thoughts ran ahead, was that he might contribute to knowledge of the nature of living stuff itself. With that ambition it was natural that he should endeavour to learn all he could about the known behaviour of living unit systems; the visible reactions, for instance, of cell protoplasm when submitted by experiment to varying conditions. There was here relatively little help from the histology of dead tissue cells as taught in the schools of anatomy and physiology. Rather had he to turn to facts revealed in the field of general biology and especially in that of experimental cytology. The remarkable potentialities of protoplasm then already being revealed by experiment strengthened the growing reaction against the mechanistic views which had held sway in the middle of the century, and stiffened the biologist in the belief that biology must remain a science *sui generis*, with its own primary concepts and its own ultimate units of significant structure. This meant the recognition of a grave breach in the unity of science, disturbing to chemical thought, and discouraging to the higher ambitions of the chemist.

The modern biochemist, rightly or wrongly, has gained in self-confidence, but it is well for him to remember that a conviction of his inability to contribute anything to the fundamental realities of biology is the basis of a definite philosophical outlook still attractive to the minds of not a few.

In an endeavour to illustrate the kind of progress biochemistry has made in my time and, if I can, its significance, I might have chosen almost any out of very many aspects. I have decided to make reference to some which to me, at least, seem characteristic. They are aspects which, in general outline at least, are among the most familiar, yet I feel that they, perhaps more than any other, have altered my own outlook. I will refer to our realisation of the fact that the phenomenon of *catalysis* is universal and fundamental in the scheme of living Nature, and further will remind you that the frank influence of molecular structure is exerted to the full in every living system. I will then ask you to remember the circumstance, now in a general sense so familiar, that the normal progress of events in every

living system calls for the presence of a number of highly specific substances, each exerting its own peculiar influence in a quantity which is almost infinitesimal; implying that the chemical equilibria on which life depends may be balanced, as it were, on pin points.

### CONCEPTIONS OF CATALYSIS

The thought of many biologists—in so far as it has concerned itself at all with the old problem as to why, in the conditions found in living tissues, chemical reactions involving stable substances proceed at all—has been content, it has seemed to me, to find a solution in the esoteric and irreducible properties of protoplasm, or in those of (to the chemist) equally unapproachable “living units” within the cell. This is not necessarily a vitalistic view, but may involve rather a disbelief in the ability of chemical methods to produce any adequate analysis. A great change in the outlook for biochemistry came, however, when it was first fully realised that the events of metabolism proceed in fact because they are catalysed, and on lines not differing in fundamentals from those of catalysis as observed in the inorganic world.

I sometimes find it hard on looking back to realise that, though the catalytic activity of enzymes outside the cell had long been known, the belief that they play so dominant a part in the interior economy of every living system was not reached till the very end of the last century. Even then the conception of intracellular enzymic activities was distasteful to many, and by not a few contemporary writers their real significance was doubted, or the extent of their influence minimised.

I will now discuss with all possible brevity certain current conceptions in the field of what is often spoken of as biocatalysis.

I must first make some reference to the colloidal structures of the cell, on which of course so many of its most fundamental properties and its visible behaviour depend.

When during earlier stages in the development of colloid science its data were applied to living systems, there was much emphasis on the general properties of surfaces and interfaces; but there was then but little understanding of how diverse and specific these properties may be. When thinking of those myriad chemical reactions (essential to the display of life) which simple molecules undergo in the aqueous phases of the cell, some of us were too apt to think of its colloidal complexes as just a special type of apparatus in which these reactions were suitably accommodated. But the recent great advances in our knowledge of surfaces, in particular of what has been called their “molecular anatomy,” which in this or that case endows a surface with quite specialised properties, has shown that the due progress of the reactions is very intimately connected with the properties of the colloidal structures. Physical chemistry and organic chemistry here meet together. The remarkable recent increase in our knowledge of the intimacies of complex protein molecules which the application of physical and

physico-chemical methods is just now providing has here to be remembered. Though colloidal, these large molecules may yet be very "active." Many, if not all, enzymes for instance seem to be specialised proteins, and the isolation of many potent plant viruses, in the accomplishment of which my colleague Mr. N. W. Pirie has had so large a share, has shown that the active unit in each case is in essentials a complex protein molecule. The view is expressed that even a chromosome may be a unit structure in the form of a large protein molecule capable of a great variety of internal steric variations!

Experimental dealings with enzymes have now produced a vast literature, but I will confine my remarks to those special catalytic agencies which promote the processes of oxidation and reduction, thus making the potential energy of foodstuffs available for the activity of living cells. Some of my own colleagues have made important contributions to this field, and on such an occasion as this I may be allowed the luxury of choosing my illustrations chiefly from results obtained by them.

#### OXIDATIONS AND REDUCTIONS

All earlier efforts to explain the occurrence of tissue oxidations were directed to the discovery of how molecular oxygen, which *vis-à-vis* the tissues is inert, is made capable of reaction. We have still to recognise the importance of oxygen activation, but it is remarkable how prominent in living cells is the additional activation of hydrogen. I must not stop to explain the steps by which realisation of this fact arose or mention the work of able investigators which led to it. In effect it means that the molecules of metabolites may be so affected by contact with special enzymes that hydrogen atoms in their structure are made labile and capable of transference to oxygen, or, and this is important, to other molecules. We now call the enzymes which so mobilise hydrogen by the name of *dehydrogenases*. It is customary and convenient to speak of the substances from which the hydrogen takes origin under their influence as hydrogen donators, and those to which it is transferred as hydrogen acceptors.

It seems sure that the influence of enzyme catalysis is never absent when other accepted criteria of life are present; or, since the present position of virus studies is making a definition of life even more evasive than it has hitherto been, let us rather say that it is never absent from a system which metabolises. Perhaps the power to metabolise independently is as good a criterion of life as any other. It is of great interest in illustration of this to follow the display of catalysis in free living unicellular organisms which, unlike the tissue cells of higher organisms, are fated to metabolise and grow in environments which may greatly vary. To the study of enzymes in bacteria, colleagues of mine in the Cambridge School of Biochemistry have made important contributions. Dr. Marjory Stephenson and (formerly) Dr. Quastel, with able collaborators, have worked there in this field for several years, and for me it would be a specially pleasant task to spend an hour in describing their researches, but I can only remark on the

significance of some of the results. I must point out, however, that many of these researches have been carried out with the use of a special technique, originating in our department but now freely used elsewhere. This involves a study of events in the external medium induced by the organisms when not growing. In these circumstances the induced chemical reactions can be much more closely studied than when growth is proceeding. For the pathologist and for classical bacteriology, the interest of researches on non-proliferating organisms is at present perhaps remote, but they reveal, as no other technique can so well reveal, how remarkable is the catalytic equipment of these minute living units.

Very striking is the great number of substances, otherwise stable, of which the molecules become activated under the influence of specific dehydrogenases on the surface of a bacterium, thus becoming potential donators of hydrogen in the sense I have mentioned. But the very same surface may also present catalytic mechanisms dealing with hydrogen on quite different lines. Dehydrogenase activity requires the presence of hydrogen acceptors for its display, but a bacterium (*Bacillus coli* for instance) may at the same time be equipped with an enzyme—a *hydrogenlyase*—which so activates the hydrogen in a molecule that it is liberated in free gaseous form; and, yet again, a *hydrogenase* which is capable of activating inert gaseous hydrogen, so that it enters into reactions as a strong reducing agent. My colleagues have shown that bacteria can so control certain chemical reactions in the external medium as to establish true thermodynamic equilibria among the reactants, a point of considerable theoretical importance. The precise means by which anaerobic organisms, those which can dispense with oxygen, and those to which its very presence is fatal, obtain their energy for growth were long obscure. The Cambridge workers have thrown most interesting light on this problem. They have shown on quantitative lines that if substances are present in the medium in suitable pairs, one capable of acting as a hydrogen donator and the other as an acceptor, the processes of hydrogen transport under the influence of dehydrogenases can itself yield sufficient energy for growth. I will mention just one other phenomenon of quite peculiar interest which my colleagues have illuminated, though they were not the first to observe its occurrence.

While certain enzymes form part of the permanent constitution of each bacterial species, it would seem that others can be newly acquired as the result of contact with particular molecules in the external medium. These have been called *adaptive enzymes* in contrast with those which are constitutional. To take an instance which in particular has been studied in my department: it has long been known that certain bacterial cultures can break down formic acid into carbon dioxide and free hydrogen. If *Bacillus coli* (say) be first grown in a broth medium completely free from formic acid, it is found, when transferred to a simple medium containing the acid, to lack all power to decompose it, whereas a culture identical in origin but grown in the presence of the acid, is afterwards found, even when not

growing, to decompose it vigorously. This is a striking phenomenon which should be borne in mind when we try to form a picture of how enzymes take origin during the growth and development of living cells in general.

There is at least some evidence that adaptive enzymes may appear *de novo* in the tissue cells of animals as a consequence of some change in the constitution of their internal environment. I do not think it over-bold to believe that here, in elementary form, we have a factor contributing to that fundamental quality of living things, the power of adaptation. It is sure that chemical adaptations, great or small, accompany all adaptations in form and function. Comparative biochemistry and all that is special in the chemistry of individual organs make this sure.

The complexity of the catalytic equipment at such apparently low levels of organisation as that of the bacteria is certainly a striking thing. Dr. Stephenson has pointed out, I think rightly, that we should not be led by teleology to believe that every one of the specific mechanisms we can demonstrate to be present in a given organism is necessarily employed in its current maintenance. It is not to be denied, on the other hand, that for free living organisms which may have to adjust to great variations in their growth media such a wide equipment has great survival value.

As you will know, much study through the years has been given to the activities of that other type of unicellular organism, the yeast cell, and to the successive chemical changes involved in alcoholic fermentation. I should have liked to dwell a little on these if only in illustration of the significant circumstance that specific catalysts can direct, as well as promote, reactions. The enzyme equipment of the yeast cell is such that, by it, sugar is converted into alcohol and carbon dioxide. On the other hand, such is the equipment of animal tissues that they convert the same sugar in similar circumstances to lactic acid. The directive influence of enzymes is an important element in their activity as contributing to the factors of chemical organisation in the cell. I must, however, say no more about the yeast cell, intensely as its activities have been studied since the days of Pasteur.

In the green plant enzymic activities are prominent and significant enough, but their organisation is much modified by the co-existence of the chlorophyll function.

#### STAGES IN OXIDATION

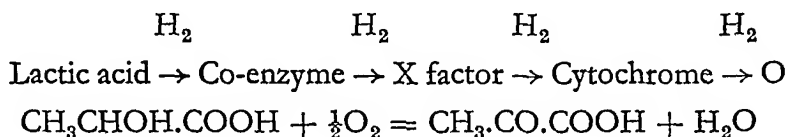
But I must rather turn to the mechanisms involved in the liberation of energy in animal tissues. Here again the mobilisation of hydrogen, and therefore the activity of dehydrogenases, is an outstanding phenomenon; but here we meet with agencies distinct from the enzymes proper, which with the enzymes form catalytic systems. Such systems may indeed be universal in biocatalysis, but they have been so far most clearly revealed in studies of animal tissues. To their study the Cambridge School, and especially Dr. M. Dixon and Dr. D. E. Green, with their collaborators, have

contributed much of importance. The following are the essentials of what we know to-day about the more typical of these systems.

It has become clear that, in the tissues, energy is not made available by a single oxidative event, but more gradually in a series of steps or stages. We picture as being first displayed the activity of the enzymes proper. These are dehydrogenases which mobilise hydrogen in the molecules of metabolites derived from food supplies. But on its way to oxygen this hydrogen finds an interrupted path. It is transferred in linked stages to a series of specific molecules which, as parts of catalytic systems, are maintained in low concentrations as permanent constituents of living cells. Each of these having acted as the hydrogen acceptor at one stage becomes the donator at the next stage; that is to say, these specific "carriers" of hydrogen, as we may call them, are capable of alternate and reversible processes of reduction and oxidation. Oxygen itself at the end of the series becomes in a somewhat special sense the final hydrogen acceptor. The dehydrogenases themselves would seem to be specialised proteins with highly specific relations. Each one can activate one particular metabolite and one only. This circumstance is highly characteristic of the systems we are considering. In many of these systems, and perhaps in the majority though not in all, the first hydrogen acceptor in the series has relations with the enzyme which are particularly intimate. It is known as a *co-enzyme*. We know of two such at least, each with specific relations. The essentials of their molecular structure are now fortunately known, and their study is dealing with a growing point in our knowledge of cell catalysis.

Another carrier (to be mentioned later) varying in nature in different systems may intervene between the co-enzyme and the penultimate member of the transport series, which is the cytochrome *c* of Keilin. This most interesting cell constituent is an iron-pyrrol compound related to blood pigment, though exercising so different a function. It is capable, like the other carriers, of reversible reduction and oxidation, and these processes involve a change of spectrum which fortunately can be observed during the life of the cell. Now the combined activities of the agencies previously mentioned reduce the cytochrome molecule with the hydrogen they transport, and the latter is then oxidised by the oxygen of respiration, though for the accomplishment of this yet another factor is required. The molecular oxygen must first be activated, and this is brought about by an agent which I think should be known as the cytochrome oxidase.

The following diagram sums up the relations of all these factors in a particular case. It illustrates the oxidation of lactic acid.



It is clear then that for mobilising the hydrogen of diverse metabolites

there are equally diverse mechanisms, and diverse paths along which the hydrogen is conducted to a place of meeting with active oxygen. It is of interest to note that some of these paths converge to join a final common path to oxygen provided by cytochrome. All this complexity gives us a glimpse at least into the organisation of events in the cell. If the only activation involved were that of oxygen itself, biological oxidations could not be so regulated, so specific, and so organised as we know they must be. Moreover, the gradual liberation of energy in successive stages, rather than only at a stage involving the union with oxygen, would seem to be a related aspect of biochemical organisation.

You will realise that I have told you only a part of a large story. In dealing with the oxidation of hydrogen, I have omitted for instance all mention of the origin of the respiratory carbon dioxide. I find I must leave that side of the story without discussion.

A tissue which in particular has occupied the labours of many biochemists, among them my colleague, Dr. Dorothy Needham, is muscular tissue. Indeed, the labour spent on revealing the nature and sequence of the chemical events within muscle-fibres has been prodigious. The facts won are so numerous that I must be content to give them the barest reference. They relate chiefly to the successive changes which set free that proportion of the potential energy contained in the carbohydrate stores of the muscle, which is liberated in the absence of oxygen. A number of specific enzymes are concerned, but what the study of muscle has in particular revealed is that certain organic molecules only interact and only undergo certain kinds of enzyme-catalysed changes when they exist in combination with phosphoric acid. The transport of the phosphoryl group from molecule to molecule and the probable simultaneous transfer of energy from exothermic to endothermic reactions, though it does not occur in the carbohydrate metabolism of all tissues, as was first shown by Dr. E. G. Holmes in a study of brain tissue, is a biochemical phenomenon of great significance.

I will refer to studies of enzyme catalysis in just one more field, that namely of embryology. The writings of Dr. Joseph Needham awakened some years ago much general interest in chemical embryology, and his own work with that of his helpers has contributed much to knowledge of the subject. It is destined to become a very fertile field. I have hitherto made no reference to developments in experimental methods, but may refer here in parenthesis to the great benefit biochemistry in general has received from the development of accurate micro- and, so-called, ultra-micro-analysis. The ability to employ with confidence minute amounts of material in quantitative experiments has made possible the solution of many problems which at one time would have been thought to be unapproachable. I have, for instance, derived much pleasure from seeing Dr. Needham, with his colleague Dr. Boell, establish significant differences in the respiration of different parts of the gastrula of the embryo by the use of a method in which only a tenth of a milligramme or less of material was employed.

I have so far put before you in barest outlines a few cases illustrating at least the kind—though by no means the extent—of our knowledge concerning mechanisms without which every biological unit would be a static instead of a dynamic system, and a dead system rather than a live one. We have much more to learn concerning the precise nature of intracellular enzymes and their mode of action, but I am sure we are on the right path for reaching that knowledge, and I will again remind you that I myself belong to a generation which began with little or no understanding of such mechanisms; to one like myself the knowledge we now possess seems quite newly won and remarkable even in its present extent.

The aspect of biocatalysis which I will finally emphasise is its specificity, which, though not absolute, is a quality persistently revealed by experiment. Whatever theory of enzyme action we may hold it is sure that the primary happening is the formation of an association between catalyst and substrate, involving some definite relation between structure in or on the former and the molecular structure of the latter. It is this dominant influence of molecular structure in living systems that I now wish to illustrate further.

I remember that not many years ago a well-known biologist claimed, in a book dealing with cytology, that as protoplasm cannot be an ordinary chemical compound, it can exhibit no molecular structure in the sense understood by the chemist. I used not infrequently to hear somewhat similar views expressed. The former part of the above claim is undoubtedly true. The use of the term protoplasm may be morphologically justified, but chemically it denotes an abstraction. It is sure that it is made up of parts in which the influence of molecular structure as understood by the chemist is all potent.

You will, I think, all realise that when physiologists revealed the existence and functions of hormones they not only gave increased opportunities for the activities of biochemists but in particular gave a new charter to biochemical thought, and with the discovery of vitamins that charter was extended. I would suggest also that the revelation of what we have come to know as the humoral transmission of nerve impulses is a confirmatory clause in that charter, and such, no less, is the recognition of "organiser" functions in the realm of embryology.

We have gradually come to learn the nature of a number of substances of relatively small molecular weight, not forming part of the colloidal structures of the tissue, though able to establish temporary relations with these, but existing in true aqueous solution, and so capable of transport in the aqueous phases of individual cells, or in the circulatory fluids of animals and the sap of plants. We know that the properties of each one of these—and they are surprisingly many—may make its influence absolutely essential for the normal progress and organisation of events in this or that living tissue. In their absence disorganisation follows. Characteristic of all these agents is that they function efficiently in amounts which are extraordinarily small.

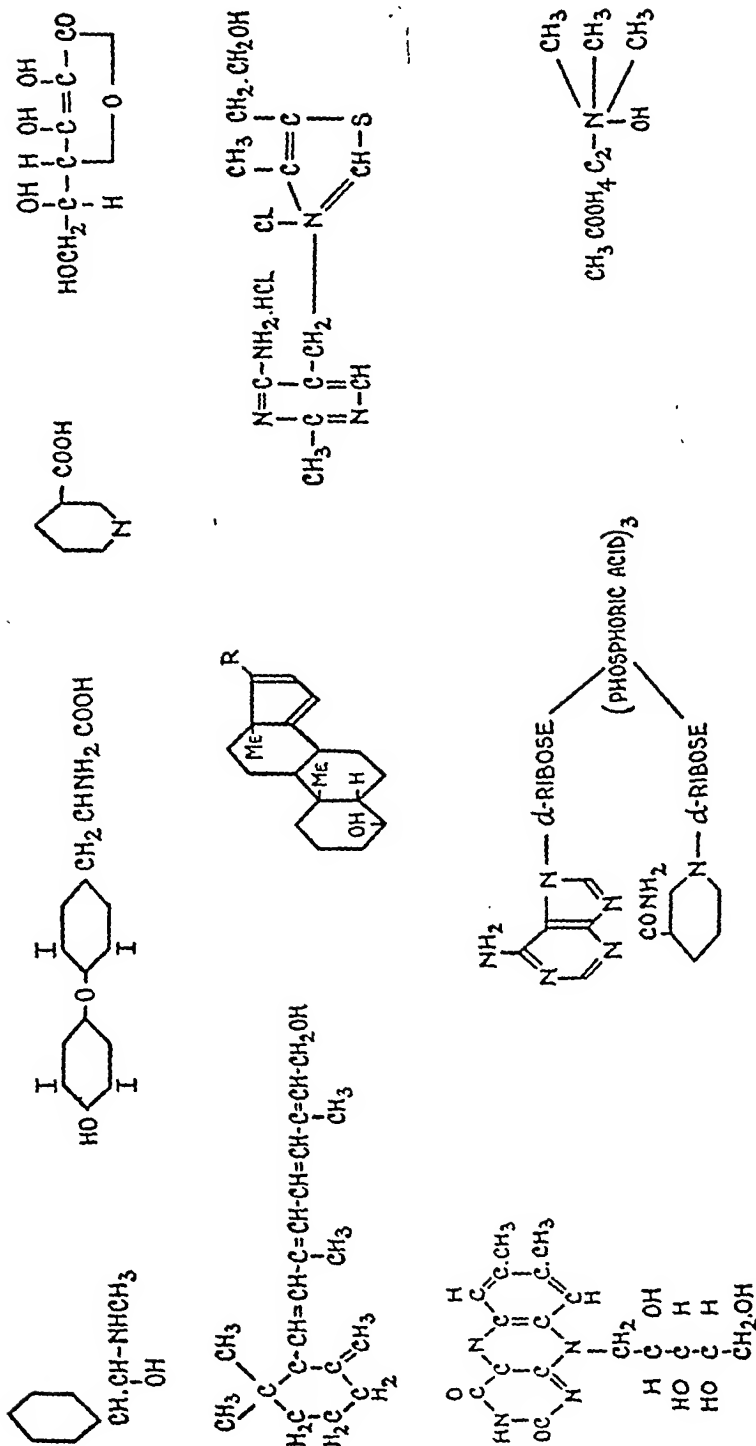


discussion of their constitution or their individual functions, nor is this necessary for my purpose, though I will presently, with a particular intention, briefly indicate some relations between constitution and function in the case of a few. In the accompanying illustration I have ventured to assemble the structural formulae of all these influential molecules so far as they are known (some dozen in all), not wishing however that the significance of structural details should trouble those who are not organic chemists, but solely to make clear to the eye what for my present theme is so important—namely, the circumstance that great diversity of function goes with a corresponding diversity of structure. It should be remembered that the influence of substances, potent in function while infinitesimal in amount, is not confined to the tissues of the higher animals. Some micro-organisms at least need what may fairly be called a vitamin supply in their growth medium; hormones necessary for growth, of which the chemical nature is now known, function in plants; and some cases of similar influences have been brought to light in the economy of invertebrates, especially interesting being that of certain insects in which growth and metamorphosis are conditioned by hormones produced by a gland situated in the head (V. B. Wigglesworth). Such functions it would seem enter widely into the scheme of Nature.

To determine just how these substances exert their potent influence is an immediate task for the biochemist. It is no simple task and has only recently revealed itself as an urgent scientific duty. It has therefore not yet gone far. It is I think sure in advance that their indispensability must be due to the circumstance that like enzymes (though on different lines) they play a dominant part in maintaining the due progress of dynamic events. We have at least some direct evidence for this.

As many here will know, when a man or animal is deprived of a supply of vitamin B<sub>1</sub> the primary effect is disorganisation of events in the nervous system. Now during the stages involved in the normal breakdown of carbohydrate in the organism the substance pyruvic acid makes a significant appearance. Professor Peters at Oxford has shown that the brain tissue of an animal which has suffered from a deficiency of this vitamin, unlike the healthy brain, is unable to deal normally with pyruvic acid, which therefore accumulates in the tissue. Experiments *in vitro* show that most minute amounts of the vitamin, acting perhaps in some essential combination, restore the power of brain tissue and other tissues which lack it, to decide the fate of pyruvic acid. This and other evidence indicates that one function of vitamin B<sub>1</sub> is to play an important part in the control of carbohydrate metabolism.

It has recently been revealed in dramatic fashion that the dire disease pellagra, so prevalent in certain countries, is due to the lack of a substance which has a relatively simple constitution—namely, nicotinic acid. In most minute amounts this can prevent or cure the most severe symptoms of this disease. Nicotinic acid must now be placed in the category of essential vitamins. I will here ask you to recall what I said about the functions of



Above are assembled the structural formulæ of a group of substances each of which, owing to its specific chemical nature, plays some dominant part in maintaining the integrity and due progress of dynamic events in living organisms. They are assembled not for insistence on the significance of structural details but only to make clear to the eye how great diversity of function is associated with equal diversity of constitution.

- (1) Adrenaline; (2) thyroxine; (3) nicotinic acid; (4) vitamin C; (5) vitamin A; (6) calciferol, one form of vitamin D, the sterol ring system here shown is also the basic structure of the sex hormones; (7) vitamin B<sub>1</sub>; (8) vitamin B<sub>2</sub>; (9) co-enzyme II; (10) acetylcholine. [Reading from left to right and top to bottom].

co-enzymes as contributing to the transference of hydrogen to oxygen. Now to the relatively complex structure of co-enzymes the structure of nicotinic acid contributes, forming that part of the whole molecule which is directly concerned in the transport of hydrogen. It would seem as though the ring structure of nicotinic acid is one which the tissues cannot synthesise, though it has important and biochemical functions to perform. It must therefore be supplied in the food. Again there is a vitamin B<sub>2</sub>, or lactoflavin, which is known to be necessary for animal growth. The molecule of this also contains a special ring structure—the alloxazine ring—which again the tissues seem unable to synthesise; hence the need of this vitamin. Now when in metabolism lactoflavin becomes linked up with a specific protein, it also plays, at least in certain cases, a notable part in hydrogen transport, the alloxazine ring being the essential carrier. These few cases show that the influence of vitamins may be exerted amid the dissimulative events which liberate energy; perhaps in the laboratory we have so far only found it in such connections because it is chiefly there we have looked for it. It seems to me sure, however, since a lack of any one among hormones and vitamins involves such profound and specific effects on tissue cells in the animal body, that their influence in a chemical sense goes deeper than this, and is somehow concerned with the organisation of reactions in the tissues. In some key positions they must exert a wide control over events.

I have put many facts before you, but they are a very small sample of those available with similar bearings. Many are facts of an order or kind which not so many years ago would have seemed beyond our power to reveal. What for the moment I would emphasise is that they are so frankly chemical that only chemical language can deal with them. Will any intellectual synthesis ultimately make easy a translation into biological terms? What degree of such synthesis may we hope for in the future?

#### STUDY OF EVENTS IN ISOLATION

I have more than once referred to “organisation.” The very word gives pause to the biochemist. If his ultimate aim is to describe adequately a living system in chemical terms, he must face the fact that he is proposing to explore a field in which chemical events are organised with a complexity not thought of in the development of classical chemical science. There are doubtless “higher” levels of organisation in the description of which chemical language can take no direct part. But if we speak of higher and lower levels, we must not do so with the assumption that one is more important than another.

Description at any one level alone will be found to have inherent limitations. At the chemical level many recent studies, apart from those on micro-organisms, have been made on intact tissue cells during true survival life *in vitro*; but those which have thrown most light on the nature of the controlling mechanisms have involved the isolation of such mechanisms.

*We have isolated nevertheless not structural materials alone but events in progress, events proceeding in space and time, and therefore, as samples, retaining something at least of the nature of the whole.* We realise, of course, that events studied in isolation may be modified when proceeding in the environment of the whole. Our methods of isolation do not, however, create artefacts, though they may lead us to study potentialities rather than actualities. It is part of our task, present and future, to distinguish the latter amid the former.

Let it be frankly admitted that biochemical studies have still before them, save in small, though not negligible, part, what I will again claim as their legitimate aim and major task—the endeavour namely to describe biochemical events from the standpoint of their organisation during life. If and when such effort succeeds, and only then, we shall know just how far chemical thought can reach towards a full description of any living unit. Many, and, I think, most to-day, believe that the full description will call in addition for thought of another kind. Nevertheless, at any one level of organisation description may have its own measure of completeness.

With regard once more to the present position of biochemistry, let me again remind you that it is only during the last few decades that its pursuits have revealed something of the nature of the individual events which must share in the organisation, and made clear how great is their number. Progress is accelerating and a young science need not fear the law of diminishing returns. Moreover, as a borderland science, apart from its own momentum, it is constantly gaining impetus and fresh opportunities from advances made on each of its frontiers. Especially is this true at the present moment.

### BIOCHEMISTRY AND BIOLOGY

I chose for this lecture an ambitious title which needs some excuse. You will have realised by now that its terms were meant to apply to a field for thought which, though surely all-important and one which, occasionally at least, must occupy the mind of every biologist, is yet, for discussion, a limited one. There are, of course, and always will be, great fields of biological endeavour, where no direct contacts with chemical thought are sought or called for; there are equally wide fields where chemical endeavours have no concern with biological thought. It is especially for those who feel an irresistibly strong desire to learn what can be known about the fundamental nature of living stuff, to realise that here two modes of thought make immediate contact. As Professor James Gray has said, "cytology may rightly claim to be the frontier state in the biological commonwealth, for within its borders biologists and chemists find common ground." Are their differences such that their thought about the living cell must be mutually exclusive; may they not rather, without either losing its own special character, prove eventually helpful, and even essential each to the other?

I was a little disconcerted when asked, after the title of this lecture was announced, how I would define biological thought for my purpose, for it

has many varieties. What I meant by it as applied in the particular field which I have made the special concern of this lecture, is such thought about a living unit which ignores, or minimises the importance of, those molecular events which must underlie the visible manifestations of its life; an attitude of mind often based perhaps on a doubt whether any attempt to correlate the molecular with the visible is within the competence of experimental science. Let me add that I have met the same doubt in the minds of some few highly competent chemists. Their view is that biochemistry should endeavour to follow every path that will lead to utility. Such paths are many and open; let us, they say, do what we know we can do and not follow a will-o'-the-wisp. I cannot agree with this view.

There are however biologists who, with none but friendly feelings for biochemical effort, and by no means excluding its results from their thoughts, yet so fully realise the complexities which their own work continues to reveal that they instinctively recoil from chemical approaches that seem too facile.

There is one who, I know, sometimes feels this whose opinion all very greatly respect and value. I mean our own professor of zoology. In his admirable book on experimental cytology, to which I personally owe much, Professor Gray, while displaying full sympathy with biochemical endeavour, to which indeed he has himself contributed, shows himself rightly concerned to warn his readers against such chemical discussions of the subject as appear to him crude. He will, I know, not mind my quoting some of his words. At one point, irritated perhaps by something recently read, he speaks out sharply. Having discussed some obscure aspects of cell structure he adds "but the position is dark, and placidly to regard the cell as though its mysteries were all but revealed by the magic wand of chemistry, is a sorry tribute to biological facts." And again, "a biological problem disguised by the sparkling terminology of the chemist is too often a pathetic and rather disreputable object." I would fain believe that the chemist—the would-be biochemist at any rate—has modesty enough to disclaim alike the possession of a magic wand, and the ability to speak a language which sparkles, but I quote these words of our distinguished colleague, realising that he is really much more merciful than they suggest, because they give me an excuse for suggesting that there are problems, essentially I think biological, which are disguised by any language which is not chemical.

I do not think in illustration of what I mean I need do more than repeat myself. With all the events that can be directly observed in living cells, including those truly remarkable occurrences involved in cell growth and division, cell differentiation in development, and the like, which it has been the privilege of classical cytology and especially experimental cytology to reveal, there must be associated molecular events as varied and in some way as controlled as are the visible events themselves. An understanding of these latter presents a problem which, however difficult of approach, is a real problem, and, surely, a biological problem. Only a frank disbelief in any real correlation between the invisible and the visible can remove

it from that category. Yet if it is to be discussed it must be in the language of chemistry. Let me just add that there is a view sometimes expressed which I think receives a measure of approval from Professor Gray. It claims that in cytological phenomena the influence of form may be more important than that of composition. If one could think only of static composition this might well be admitted, but biological components are seldom static, and dynamic changes, if they are specific, must be undergone by specific materials. In any case it seems to me that the view fails in certain ultimate issues. It is suggested for instance that the form of a chromosome rather than its chemical composition accounts for its influence. *But at such magnitudes form, as ordinarily understood, merges into molecular structure.* Form holds its own, but as molecular form, the shape of molecules, and of this we have knowledge much more objective than was the case a few years ago. As I earlier mentioned, some leading cytologists are even coming to the conclusion that a chromosome is a unit structure and a complex protein molecule. I am not qualified to judge how far such a concept is justified, but if it be so it supports my general theme.

I fully sympathise with the distaste of biologists for facile chemical explanations which are *a priori*, and based, as Professor Gray says, on "hypothetical biology." To read their protests is indeed a desirable discipline for the mind of the chemist. I think however that if chemists have sometimes so offended they—professed biochemists at any rate—are much less likely to do so to-day. They are at any rate, be it noted, concerned to offer descriptions rather than explanations.

Professor Herbert Dingle, in his noteworthy book, *Through Science to Philosophy*, referring to a certain conclusion of his own, remarks, "in short, on looking forward with hope to a time when physics and biology will be safely wedded, it suggests that physics should be the proposer who, on bended knee, begs for acceptance, but it does not presume to suggest what name the happy couple should take." Under the head of physics he was doubtless thinking of physical science in general. I should like to find among my audience agreement with my own belief that biochemistry is to-day mature enough to justify such a humble proposal for matrimony, and trust you will share my hope that biology, in so far as it has been coy, will not remain so.

#### A SCIENTIFIC BORDERLAND

It is an old saying, abundantly justified, that where sciences meet there growth occurs. It is true moreover to say that in scientific borderlands not only are facts gathered that are often new in kind, but it is in these regions that wholly new concepts arise. It is my own faith that just as the older biology from its faithful studies of external forms provided a new concept in the doctrine of evolution, so the new biology is yet fated to furnish entirely new fundamental concepts for science, at which physics and chemistry when concerned with the non-living alone could never arrive.

Whether they will prove to be such as to create an intellectual revolution comparable with that due to the development of atomic physics, who shall say? I sincerely believe that such new concepts are there to be revealed, but only perhaps as the result of new intellectual interactions. I speak, as you realise, with a special brief, but I think it is worth your while to consider whether a new line of advance in biology (not of course to the exclusion of many other lines) may not come from researches motivated by a full conviction that each observed event in the complex behaviour of a living system is associated with equally complex and, surely, equally biological, events at the molecular level of its constitution; and by the further conviction that both its aspects are worthy of equal intellectual concern. We must specialise in our methods, but we should share our interests.

You may ask whether I have in mind any policy which would encourage the intellectual approaches I have been advocating. It may be that they would be helped in the future by some modifications in present educational policy, but I will not enlarge on such a matter here. The main thing to hope for is the establishment of favourable mental attitudes.

In concluding my remarks I would like to take this exceptional opportunity for expressing a hope sincerely felt. As one shortly to leave the field of biochemical endeavour, I hope that my younger friends and colleagues will always justify their special designation and strive so far as is humanly possible to be biologists as well as chemists. As an ideal this is not easy of attainment, as I know well; but every effort towards it will increase the interest and the value of the work before them.



1943.

"I think we may say that organic chemistry, moving closer to physics, is now passing from its classical period into a romantic one. . . ." (p. 203).



## Selections from “Brighter Biochemistry”



## SELECTIONS FROM "BRIGHTER BIOCHEMISTRY"

THIS book would not, it is felt, be complete without a few extracts from the comic journal of the Sir William Dunn Institute, which was brought out annually for a period of eight years from 1923 onwards. There was probably not a single member of the staff, whether research worker or technical assistant, who did not contribute something to these volumes, and many of the verses, short stories, drawings, or parodies of scientific papers, were really witty and amusing. Those who edited this journal (which found a ready sale in the University, especially among the medical and other students attending courses in the Institute) were:

- 1923-24. J. H. Quastel and Margaret D. Whetham (later Mrs. A. B. Anderson)
- 1925-26. M. D. Whetham, M. G. L. Perkins and Dorothy M. Needham
- 1926-27. W. R. Wooldridge and Barnet Woolf
- 1929-30. C. A. Ashford and L. H. Stickland
- 1931. K. A. C. Elliott, D. R. P. Murray and A. Leese

After that date, as clouds began to gather on the horizon with the course of events in Central Europe, there was a general decline in gaiety, and the journal was discontinued. Those precious copies which remain, however, still bear witness to the spirit of the Institute during the years which were perhaps the zenith of Sir F. G. Hopkins' life, when he was at the head of a large Institute which he had himself created, and which was full of brightness, not only of intellect and experiment, but of comradeship, alive awareness of the world outside biochemistry, and warm inspiration owed and universally acknowledged to the leader and founder.

Here we reproduce (*a*) a versified "Report to the Secretary of the Sir William Dunn Trustees for the year 1924-25," by J. B. S. Haldane, (*b*) a cartoon on the occasion of Sir F. G. Hopkins' knighthood, by Barnet Woolf, and (*c*) a contribution by Hopkins himself, "Biochemical Equipment: a Glance into the Future." This last piece is particularly interesting, since it harks back to the days of his first work in Cambridge, "Aminophilus" standing for himself, and "Collophilus" for Sir W. B. Hardy, who had at the same time been making his fundamental researches on colloid solutions.

(a)  
REPORT  
TO THE  
SECRETARY OF THE  
SIR WILLIAM DUNN TRUSTEES  
FOR  
THE YEAR  
1924-1925

---

Sir, on the upper floor the classes  
Included genii and asses,  
The former got out tryptophane,  
The latter poured it down the drain.  
The well-known author, Mr. Cole,  
(We hope again to see him whole)  
Besides the classes that he took  
Re-wrote his admirable book;  
Next door the firm of Seth and Luck  
Found that ammonia comes unstuck  
From carbamide in pigs and pugs  
As easily as in beans and bugs.

Professor Hopkins down below, too,  
Has measured the uptake of  $O_2$   
By proteins from every organ  
In Barcrofts, watched by Ruse and Morgan;  
The contents of our cranial domes  
Were studied by the spouses Holmes,  
Who find each intellectual gent. owes  
His mental powers to a pentose.  
Miss Robinson and R. McCance  
Have made a notable advance  
In dealing with tyrosinase,  
And the queer laws which it obeys.  
Aided by Anderson and others  
Our saccharologist Carruthers  
Attacked the problem of rotation  
Of glucose during activation.  
The indefatigable Harrison  
Found out how glutathione carries on  
Quite free from iron, in all sorts  
Of lovely gadgets made of quartz.

I cannot synthesise a bun  
By simply sitting in the sun;  
I do not answer "Yes, yes, yes,"  
If I am offered meals of S;  
I must admit I always flee  
When offered drinks of  $\text{NH}_3$ ;  
I fear that  $\text{NaNO}_2$   
Would turn my haemoglobin blue;  
And you are really quite mistaken  
To give me nitrate, save in bacon;  
The synthesis of tryptophane  
My tissues find too great a strain;  
And I metabolise no more  
On breathing things like  $\text{CH}_4$ ;  
While even Barcroft, as you know,  
Could never oxidise CO;  
No men, no guinea pigs, and few crows,  
Can make a simple thing like sucrose;  
But readers, rhizostomes and rats  
Are fairly good at making fats.  
So I shall concentrate on this,  
My most effective synthesis,  
And on my arm I'll keep a spot shorn  
For venepuncture by Miss Watchorn.

Zoophilists need never feel  
Much pity for the dogs of Hele  
(Even for those whom Nature dooms  
To drinking compounds made by Coombs);  
Although they sometimes look dejected,  
Their barking powers are unaffected.

I next pass on to Mrs. Onslow,  
Whose knowledge lays all mere male dons low.  
I know I'd rather meet a lion\* in  
My path than talk on anthocyanin.  
I am afraid the Lab. has lost a  
First-rate researcher in Miss Foster;  
A pancreatic anti-ferment  
Came quite near causing her interment;  
Her ether (she was not to blame)  
Exploding far from any flame.

\* Or leap from some queer crag or funny cliff,  
Or smell mercaptans made by Tunnicliffe.

Attached hereto there is a pastel\*  
 Portraying Dr. J. H. Quastel  
 Surrounded by his bugs protesting  
 Against the work they're given when resting.  
 Wooldridge and Woolf (who will not rhyme)  
 Assisted in this sordid crime.  
 Still harder were the problems set 'em  
 By Misses Stephenson and Whetham;  
 For data on how their bugs feel  
 Ask Timothy† (not Dr. Hele).  
 Assisted by Miss Sylva Thurlow  
 (Now, I regret to say, on furlough)  
 Our demonstrator, Dr. Dixon,  
 Tried several entertaining tricks on  
 Catalysis of linked reactions,  
 A subject which the vulgar hack shuns.  
 Thanks to some work of Mr. Hill's  
 Iron is banished from our pills;  
 Pale people wishing to be pink  
 Can make fresh pigment out of zinc,  
 Of, if their doctors think it proper,  
 Iron may be replaced by copper.

Our Mr. F. J. W. Roughton  
 Has watched his haemoglobin spout on  
 Its colour changing as it goes  
 From purple to a lovely rose.  
 I should be worse than a barbarian,  
 If I omitted the Librarian;  
 The ways impartially she probes  
 Of publishers and anaerobes.  
 He would be an extremely bold 'un  
 Who'd steal a tube from Mr. Holden;  
 Armed with a bludgeon and a scimitar  
 He sits and guards the polarimeter.  
 What Winter got from all those goats  
 Is no fit subject for my notes;  
 Nor shall I soil these pages with  
 The horrid things found out by Smith,  
 Because Part I must never know  
 How glucose sends blood-sugar low,  
 While insulin may make it rise  
 To everybody's great surprise.

\* Omitted for lack of space, Eds. † The Timothy-grass bacillus.

The work of Messrs. Kay and Irving  
To me, at least, is most unnerving;  
Since oxalate makes glucose leak in  
To corpuscles, despite my shrieking  
They want to centrifuge my veins,  
And see how much the cell contains.  
The victims of Havard and Reay  
Ran up and down the stairs all day,  
And each became an expert bleeder  
Under the influence of the Reader.

I now descend below the floor,  
Passing the Prep. Room and the Store,  
To where the ever-patient Perkins  
Studies the very curious workings  
Of *Saccharina* which destroys  
The theory that boys will be boys;  
And Thomson gets out various dyes  
From wings of moths and butterflies.  
I often wonder into which hell  
S. Coleridge would condemn our Mitchell  
For playing interesting tricks  
Suggested by the fiendish Hicks  
Upon the nitrogen partition  
Of rats with copious imbibition.  
Next I proceed to sing the Murrays;  
One dealt with the Professor's worries;  
The other roasted unhatched chicks  
Next door to where the rats of Hicks  
Ran round and round in little wheels  
Between their tryptophaneless meals.  
Then N. J. T. and D. M. Needham  
Transfix amoebae, yes, and bleed 'em,  
Besides determining their buffering,  
And thus preventing human suffering.  
Although this year there's little to be  
Remarked about the rats or Ruby,  
In next year's Lab. notes, when I write 'em in,  
I hope to tell of a new vitamin (perhaps).  
So though no doubt you often hate us  
For asking for new apparatus,  
I am quite sure you will relent  
On learning how the money's spent.

(b)



He was a verray gentil parfit Knight. G. Chaucer.

Cartoon on the occasion of Sir F. G. Hopkins' Knighthood,  
by B. Woolf.

(c)

"BIOCHEMICAL EQUIPMENT—A GLANCE INTO  
THE FUTURE"

## I.

A.D. 1898.

[*A small room in the building which still adorns Corn Exchange Street.*]

*Collophilus*: Pray lend me your test tube, *Aminophilus*; my own is under repair.

*Aminophilus*: Most certainly, *Collophilus*; I know that it will receive your customary care in the handling of apparatus. You find me, as it happens, in a philanthropic mood; Charles has just captured a mouse in the cellar.

*C.*: Felicitations! You will at last be able to proceed with your Nutritional Investigations. Let me confess that I, too, am in expansive mood. Before leaving my house this morning, I was able to purloin an egg (I think a fresh one) from the domestic store. I may say that the yolk, and part of the white, are very heartily at your service.

*A.*: Too generous! I am, it is true, in need of a source of amino-acids; but nothing must interfere with your important Colloidal Studies.

*C.*: Believe me, it is impossible for me to employ, at the moment, all the material represented by a whole egg. The Laboratory, I find, lacks any source of polyvalent ions. I possess sodium chloride, which, like the egg, is of domestic extraction; but can you, I wonder, provide me with a minimal quantity of, say, ammonium sulphate?

*A.*: Fortunately, owing to the generosity of the Professor of Agriculture, I possess a specimen of that useful salt. A portion is at your command.

*C.*: Encouraging in the very highest degree! You embolden me to mention, though with much hesitation, my need for metallic ions of high valency. I possess a solution of pen-nibs in hydrochloric acid, but—

*A.*: If, my dear *Collophilus*, you are venturing to think of salts, of, let us say, Cerium or Thallium (I mention them with awe), then indeed you are, as you claim, in expansive mood. We must, you know, be reasonable. May I suggest a possibility? I am thinking of the domestic store. Could you not try alum?

*C.*: An inspiration! To-morrow the coagulation potency of a trivalent ion shall be explored.

[*On the morrow Collophilus opens a new Chapter of Science.*]

## II.

A.D. 1925.

[*Various localities in a building which (when it tones down) will adorn Tennis Court Road.*]

*A Voice:* Those old research Johnnies had a soft time, you know; couldn't move without walking into some easy job or other.

*Many and Diverse Voices:* Seven goats on Tuesday . . . two hundred and fifty hydrogen donators; especially some with an asymmetric sulphur atom. . . . There ought to be more microbalances. . . . Forty sheep's brains as soon as you can. . . . Try a hafnium electrode. . . . Fancy, only one mercury vacuum pump! . . . and no micro-spectrophotometer! . . . The little creatures must have prolin; heaps of it. . . . A statistical study is the only thing; inject twelve rabbits a day with thyroxin for six months. . . . Why is there never a Barcroft free? . . . We're injecting ionium salts into the nucleus. . . . Why doesn't the Cold Store get a bit nearer to absolute zero? . . . [*Editors:* Only he who formerly purloined the egg can say.]\*. . . I got hold of all Kahlbaum's stock, but yesterday the Reader swallowed the lot. . . . Why should she run *all* the centrifuges *all* day? . . .

*A Voice* (formerly employed to encourage the mouse): I must have standard rats; and please order a ton of yeast.

*The Voice of the Comptroller:* Steady on, Professor; remember our economy stunt. By the way, how about that new animal house for the dogs?

*The Voice of Olive:* Tea's ready.

*The Voice of Charles* (who formerly caught the mouse): THESE RESEARCHERS . . . !

(But "These Researchers" are filling up the *B.J.* with jolly good stuff, and are preparing the way for A.D. 2000.)

## III.

A.D. 2000.

[*In a range of buildings occupying Parker's Piece.*]

*Senior Superinvestigator:* Now that Biochemistry has become an exact Science, one realises how superfluous are these buildings and their equipment. It is with Brains and the Biocalculus that progress is now made. We must, I feel, resign these Laboratories to the physicists, who, of course, have got to begin their experimental work all over again. What a pity the biocalculus does not apply to the crude phenomena they have hitherto studied! It is well that we broke up that "Cycle of Definitions" that old

\* Sir W. B. Hardy had become Director of the Low Temperature Research Station at Cambridge.

Eddington used to talk about. By the way, you are yourself too prone to trifle with experiments. You must restore your historical sense by reading your "Needham" again; the twenty-second edition, 1927, is the best.

*Junior Superinvestigator:* But surely there are experiments still worth making. I found yesterday, for instance, that gastric hydrochloric acid contains exclusively the heavier isotope of chlorine. I find no mention of this fact in our automatic wireless records.

*S.S.:* Nevertheless, do not be in a hurry to broadcast it. Such selective activity on the part of the gastric pseudo-psychoids could have been predicted from first principles.

*J.S.:* I also calculated yesterday that the condition of glutathionuria, which the Harley St. Iatrochemists describe as being now so common, must be due to perturbation in the hexagonal orbit of the fifteenth hepatic pseudo-psychoid.

*S.S.:* That's interesting; but queer! It seems to contradict Ratinski's equations. If I were you, I would have the literature explored. The eleventh recording centre at New York specialises in that particular pseudo-psychoid. Get in touch with them, and report at lunch time.

F. G. H.



# Roster of Sir F. G. Hopkins' Collaborators and Colleagues



## ROSTER OF SIR F. G. HOPKINS' COLLABORATORS AND COLLEAGUES

THE following roster is essentially a list of all those who worked in the Cambridge Biochemical Laboratory since its foundation at the beginning of the present century. One of the difficulties of compiling it has been that it is hard to draw sharp lines of distinction—thus Sir F. G. Hopkins retired in September, 1943, but continued to take an interest in the work of the laboratory for a considerable time afterwards. The list is not therefore brought sharply to a stop at that point. In this connection the following signs, of which we have availed ourselves in the roster, need explanation:

- (Collab.) There were certain cases in which an investigator, though not himself actually working in the Biochemical Laboratory, was associated very closely with work going on there, and published a series of collaborative papers with members of the Laboratory. Such names are qualified by this sign.
- (Nutr.) The Dunn Nutritional Laboratory (University of Cambridge and Medical Research Council), springing as it did from one of Sir F. G. Hopkins' major research interests, and united as it has always so closely been by ties of friendship with the parent Laboratory, has been included in the roster. Qualification of a name by this sign indicates that the person spent part or all of his time in Cambridge at the Nutritional Laboratory.
- (Private) During the earlier years of Sir F. G. Hopkins' residence in Cambridge, there were a few scientists who, while closely associated with him, owned, and worked in, private laboratories. Hence the use of this sign.

The difficulty of drawing up the present list has been largely due to the deficiencies of reference books. Even membership lists of societies give only last known addresses, and often not so much. Three copies of the list were therefore circulated to some thirty present and past members of the Laboratory, and many of the details given were inserted by them. The list was assembled in the first place from the index cards in the laboratory office, from the laboratory photograph record album, and from the annual volumes of collected papers.

One of the lines of distinction most difficult to draw has been that between the Biochemical Laboratory and friends in other Cambridge institutes whose work was closely linked with that of Sir F. G. Hopkins

and his immediate colleagues. In this connexion it may not be out of place to mention here Professor D. Keilin, F.R.S., and the brilliant team which he has for so many years directed at the Molteno Institute. There were also the biochemists at the Low Temperature Research Station and in the Botanical Laboratory, not to speak of many friends in physiology, comparative physiology, and chemistry.

The historian of modern science may find in this roster some matter of interest. It includes citizens of as many as 28 countries. It includes occupants of some 75 professorial chairs, scattered all over the world. It is able to show 24 Fellows of the Royal Society and two Nobel Laureates. Interesting from the point of view of modern trends is that in the list will be found some 25 biochemists who attained eminent positions in governmental, as opposed to the more traditional academic or industrial, science. Notable also is the presence of a considerable number of clinical or hospital biochemists. Both the League of Nations and the United Nations found willing workers from among those associated with Sir F. G. Hopkins. Such, in sum, was the inspiration and influence of a man whose characteristics, though inadequately, have been the subject of essays earlier in this volume.

It will be appreciated that for many reasons, some of which are obvious enough, it has been a matter of great difficulty to attain satisfactory accuracy in the details of this roster. All those, therefore, who may find any inaccuracies in the material here presented, either as to details concerning themselves or concerning others; or any omissions; are requested to inform the Editors as soon as possible, so that the necessary changes may be made in the next edition of this volume.

IVY SALISBURY

JOSEPH NEEDHAM



BIOCHEMICAL LABORATORY GROUP, 1916.

*Left to right—Back:* Harold Raistrick, Mrs. Cornish, Ginsaburo Totani. *Middle:* Dorothy Jordan-Lloyd, F. G. Hopkins, Mrs. Onslow. *Front:* Mrs. Bulley, Gerald Winfield, S. W. Cole.

BALLINGER, Maurice [U.K.]	.. .. .	1938
BAPTIST, Noel G. [Ceylon]	.. .. .	1946
BAWDEN, F. C., F.R.S. (Collab.) [U.K.]	.. .. .	1936-40
Head of the Department of Plant Biochemistry, Rothamsted Agricultural Experimental Station, Harpenden.		
BEATTY, Richard A. [U.K.]	.. .. .	1938-40
Department of Animal Genetics, University of Edinburgh.		
BELL, David J. [U.K.]	.. .. .	1936-
University Lecturer in Biochemistry, Cambridge.		
BENDALL, James R. [U.K.]	.. .. .	1940-42
Scientific Officer, Low Temperature Research Station, Cambridge.		
BENTON, H. Eric [U.K.]	.. .. .	1930-48
Glassblowing Technician, Biochemical Laboratory, Cambridge; later at Makerere College Medical School, East Africa.		
BERNHEIM, Frederick [U.S.A.]	.. .. .	1926-28.
Later Associate Professor of Physiology, Medical School, Duke University, Durham, N.C.		
BERNHEIM, Mrs. F.	.. .. . <i>see</i> HARE, Mary	
BERRILL, Mrs. Dorothy B. [U.K.]	.. .. .	1942-45
BEZNÁK, A. B. L. [Hungary]	.. .. .	1935-37
Later Professor of Physiology, University of Budapest.		
BHAGVAT, Kamala [India]	.. .. .	1937-39
Later Director, Coonoor Nutritional Institute, India.		
BIRCH, Thomas W. [U.K.]	.. .. . 1929-30 (Nutr.)	1930-37)
Biochemist, Department of Medicine, Western Reserve University, and Lakeside Hospital, Cleveland, Ohio (died 1939).		
BLES, E. J. (Private) [U.K.]	.. .. . <i>ca.</i>	1910
BLISS, Sidney [U.S.A.]	.. .. .	1936
Later Professor of Biochemistry, Tulane University Medical School, New Orleans, La.		
BOELL, Edgar J. [U.S.A.]	.. .. .	1937-38
Later Professor of Zoology, Yale University, New Haven, Conn.		
BOOTH, Vernon H. [U.K.]	.. .. .	1934-35
Later at Physiological Laboratory and Nutritional Laboratory, Cambridge.		
BOURGUIGNON, Mme. G. [France]	.. .. .	1925
BOURNELL, John C. [U.K.]	.. .. .	1940-
Research Worker, Agricultural Research Council.		
BOXER, George [Australia]	.. .. .	1939
BRACHET, Jean [Belgium]	.. .. .	1934-35
Later Professor of Experimental Morphology, University of Brussels.		
BRECHER, Leonore [Austria]	.. .. .	1936
BREKKE, Bård [Norway]	.. .. .	1937
BROSTEAUX, Jeanne [Belgium]	.. .. .	1936

BROWN, Robert K. [U.S.A.]	..	..	..	..	..	1932-33
BROWN, Russell [U.K.]	..	..	..	..	..	1937-
Technical Assistant in the Advanced Class, Biochemical Laboratory, Cambridge.						
BULLEY, Mrs. E. E. [U.K.]	..	..	..	..	..	1922-23
(Mrs. V. H. Mottram)						
BUTLER, Mrs. Jean M. [U.K.]	..	..	..	..	..	1935-37
Rothamsted Agricultural Experimental Station, Harpenden.						
BUNTON, B. H. [U.K.]	..	..	..	..	..	1932
CAJORI, Florian A. [U.S.A.]	..	..	..	..	..	1934
Associate Professor of Physiological Chemistry, University of Pennsylvania, Philadelphia, Pa.						
CALLOW, Mrs. A. Barbara [U.K.]	..	..	..	..	..	1919-46
Research Worker, Medical Research Council; later Librarian of the Colman Library (died 1948).						
CALLOW, E. H. [U.K.]	..	..	..	..	..	1921-22
Senior Principal Scientific Officer, Low Temperature Research Station, Cambridge.						
CAPUTTO, Ranwel [Argentine]	..	..	..	..	..	1945-46
CARROLL, D. C. [U.K.]	..	..	..	..	..	1923
CARRUTHERS, Albert [U.K.]	..	..	..	..	..	1924-27
Later Lecturer in Biochemistry, Peiping Union Medical College, Peiping, China; then Director of Research, Beet Sugar Factory, Peterborough.						
CASE, E. Martin [U.K.]	..	..	..	..	..	1927-32
Biochemical Department, Middlesex Hospital, London.						
CHAIN, Ernst, F.R.S., Nobel Laureate [U.K.]	..	..	..	..	..	1933-35
Research Worker, Sir Wm. Dunn Institute of Pathology, University of Oxford; later Professor of Biochemistry, Instituto Superiore di Sanita, Rome.						
CHIANG, Ch'ing (Peter C.) [China]	..	..	..	..	..	1928-29
Later Professor of Biochemistry, Dean of the Medical College, and President, successively, of Chilun University, Tsinanfu (died 1937).						
CHIU, Chiung-Yün [China]	..	..	..	..	..	1946-
Formerly at Department of Physiology, National Central University Medical School, Chéngtu, Sze.						
CLARIDGE, Peter R. P. [U.K.]	..	..	..	..	..	1934-36
Senior Scientific Officer, Directorate of Food Investigation, D.S.I.R.						
CLARK, A. Barbara	..	..	..	..	see Mrs. A. B. Callow	
CLIFT, F. Paul [U.K.]	..	..	..	..	..	1931-33
Biochemist, Guinness Brewery, Dublin, Eire						
CLIFTON, Charles E. [U.S.A.]	..	..	..	..	..	1936-37
Later Professor of Bacteriology, Stanford University, California.						
COARD, Joan [U.K.]	..	..	..	..	..	1933-40
Secretary.						
COHEN, Arthur [Canada]	..	..	..	..	..	1934-35

COHEN, Jacob A. [Holland]	.. .. .	1942-43
Major, Royal Netherlands Army Medical Corps, later Director of the Medical Biological Research Institute, Netherlands Defence Council, Leiden University.		1945-47
COLE, Sydney W. [U.K.]	.. .. .	1901-
Lecturer in Medical Chemistry, University of Cambridge (retired 1943).		
COLWELL, Alfred [U.K.]	.. .. .	1919-35
Technician in Charge of Pregl Analysis Room, Biochemical Laboratory, Cambridge (died 1935).		
COOK, Robert P. [U.K.]	.. .. .	1926-32
Later Lecturer in Biochemistry, University of St. Andrews, at Dundee.		1935-40
COOMBS, Herbert I. [Australia]	.. .. .	1923-27
CORNISH, Miss [U.K.]	.. .. .	1916
CORRAN, Henry S. [U.K.]	.. .. .	1937-39
COTTON, Marjorie G. [U.K.]	.. .. .	1943-44
COWELL, Alfred J. [U.K.]	.. .. .	1921-
Technical Assistant in Charge of Elementary Classes, Biochemical Laboratory, Cambridge.		
CRAMMER, John L. [U.K.]	.. .. .	1941-42
Flight-Lieut. R.A.F. Medical Service, Editor of Penguin <i>Science News</i> .		1946-47
CROOK, Eric M. [Australia]	.. .. .	1937-42
Later Senior Lecturer in Biochemistry, University College, London.		
CROSS, Mary C. A. [U.K.]	.. .. .	1935-38
(Mrs. Jewett)		& 1940-42
CRUICKSHANK, E. W. H. [U.K.]	.. .. .	1925
Later Professor of Physiology, University of Aberdeen.		
DAINTY, Mrs. Mary E. [U.K.]	.. .. .	1941-42
DANIELLI, James F. [U.K.]	.. .. .	1938-45
Scientific Officer, Marine Biological Laboratory, Plymouth; later Reader in Biochemistry, University of London, at Chester Beatty Research Institute, Royal Cancer Hospital.		
DANIELLI, Mrs. Mary [U.K.]	.. .. .	1938-45
DANN, William J. [U.K.]	.. .. .	1925-28 (later Nutr.)
Later Professor of Nutritional Science, Duke University School of Medicine, Durham, N.C. (died 1948).		
DARRAH, John H. [U.K.]	.. .. .	1943-46
DAVENPORT, Harold E. [U.K.]	.. .. .	1942-
Staff Member, A.R.C. Unit of Plant Biochemistry, Cambridge.		
DAVIES, Ronald [U.K.]	.. .. .	1942-
Staff Member, M.R.C. Unit of Chemical Microbiology, Cambridge.		
DAWSON, Charles R. [U.S.A.]	.. .. .	1938-39
Associate Professor of Organic Chemistry, Columbia University, N.Y.C.		

DIWAN, John G. [Canada]	1936-39
DIXEY, Douglas [U.K.]	1927-37
Laboratory Assistant, Biochemical Laboratory, Cambridge.	
DIXON, Kendal C. [Eire]	1933-36
Later Fellow of King's College and University Demonstrator in Chemical Pathology, Cambridge.	
DIXON, Malcolm, F.R.S. [U.K.]	1921-
Demonstrator, Lecturer, and later Reader in Enzyme Biochemistry, University of Cambridge.	
DOWNS, Helen R. [U.S.A.]	1925-27
Formerly Instructor in Biochemistry, Peiping Union Medical College, China; afterwards Associate Professor of Biochemistry, Barnard College, Columbia University, N.Y.C.	
EDDY, George S. [Canada]	1925-27
Later Professor of Physiology, Duke University, Durham, N.C.	
EDDY, Pauline E. [U.S.A.]	1927-30
Later Professor of Pathology, Western Reserve University, Cleveland, Ohio.	
ENGLE, Elizabeth [U.K.]	1935
(Mrs. T. Work)	
National Institute of Medical Research,	
ERRALL, John T. [U.S.A.]	1924-25
Later Associate Professor of Biochemistry, Harvard Medical School, Boston, Mass.	
EVON, Norman L. [New Zealand]	1934-36
Later Associate Professor of Physiology, University of Otago, Dunedin.	
ELLORI, Kenneth A. C. [South Africa]	1927-33
Research Chemist, Franklin Institute, Philadelphia; then Assistant Professor of Biochemistry, University of Pennsylvania, Phila.; later Associate Professor of Biochemistry, McGill University, Montreal, Canada.	
ELSDEN, Sidney R. [U.K.]	1936-46
Senior Lecturer in Microbiology, University of Sheffield.	
EINTEIJM, Conrad A. [U.S.A.]	1929-30
Professor of Physiology and Biochemistry, University of Wisconsin, Madison, Wis.; Editor of <i>Journal of Nutrition</i> .	
EMERSON, R. [U.S.A.]	1939
Professor of Plant Physiology, University of Illinois, Urbana, Ill.	
ERPS, Helen M. R. [U.K.]	1941-45
(Mrs. Tomlinson)	
FANEUCHEN, Isidor (Collab.) [U.S.A.]	1936
National Research Fellow in Protein Chemistry, Massachusetts Institute of Technology, Cambridge, Mass.; later Professor of Crystal Physics, Polytechnic Institute, Brooklyn, N.Y.	

FARKAS, A. [Hungary]	.. .. .	1934
FARKAS, L. (Collab.) [Hungary]	.. .. .	1934
Later Professor of Physical Chemistry, Hebrew University, Jerusalem (died 1949).		
FEARON, W. R. [Eire]	.. .. .	1922
Later Professor of Biochemistry, Dublin University, and Fellow of Trinity College, Dublin.		
FISCHMANN, Kate (Nutr.) [U.K.]	.. .. .	1933
(Mrs. D. Shoenberg)		
FLEISCH, Alfred [Switzerland]	.. .. .	1923-24
Later Professor of Physiology, University of Lausanne, Switzerland.		
FLETCHER, Sir Walter Morley, F.R.S. (Collab.) [U.K.]	..	1911
Later First Secretary of the Medical Research Council (died 1933).		
FORREST, W. D. [U.K.]	.. .. .	1923
FOSTER, Dorothy L. [U.K.]	.. .. .	1921-26
(Mrs. Palmer)		
FOX, Denis L. [U.S.A.]	.. .. .	1938-39
Later Professor of Marine Biochemistry, University of California, attached to Scripps Institution of Oceanography, La Jolla, Calif.		
FRANCIS, G. E. [U.K.]	.. .. .	1940-43
Later Lecturer in Biochemistry, St. Bartholomew's Hospital Medical School, London.		
FRIEDMANN, Ernst [U.K.]	.. .. .	1932-46
Formerly Professor of Biochemistry, University of Strassburg.		
GALE, Ernest F. [U.K.]	.. .. .	1936-
Later Director of the M.R.C. Unit of Chemical Microbiology, Cambridge, and Fellow of St. John's College.		
GALLAGHER, P. H. [Eire]	.. .. .	1923-24
Later Lecturer on Soil Physics, Department of Agriculture, Dublin University.		
GEMMILL, Chalmers L. [U.S.A.]	.. .. .	1934
Later Associate Professor of Physiology, Johns Hopkins University Medical School, Baltimore, Md.		
GILL, Phyllis M. [U.K.]	.. .. .	1935 & 1938-39
(Mrs. O'Donovan)		
GIRSAVIČIUS, Juosas O. [U.S.S.R.]	.. .. .	1928-33
GOOCH, Alfred G. [U.K.]	.. .. .	1925-27
GORDON, Arthur H. [U.K.]	.. .. .	1938-40
Carlsbergfondets Biologiske Institut, Copenhagen.		
GRAY, Frederic V. [Australia]	.. .. .	1938-39
GREEN, David E. [U.S.A.]	.. .. .	1932-40
Later at Columbia University, New York, and Professor of Biochemistry, University of Wisconsin, Madison, Wis.		
GREEN, Miss P. [U.K.]	.. .. .	1933
(Mrs. Clark)		

- GREVILLE, Guy D. [U.K.] .. .. . 1944-  
University Lecturer in Biochemistry, Cambridge.
- GREY, Egerton C. [U.K.] .. .. . 1920-28  
Nutritional Science Investigator in Japan for the League of Nations (died 1929).
- GUHA, Bireth Chandra [India] .. .. . 1930-31  
Later Professor of Biochemistry, University of Calcutta; Director of Nutritional Services, Government of India, and First Counsellor in Agricultural Sciences, Department of Natural Science, UNESCO.
- HALDANE, John Burdon Sanderson, F.R.S. [U.K.] .. .. . 1923-32  
Sir Wm. Dunn Reader in Biochemistry, Cambridge; later Professor of Biometry, University College, London.
- HALL, Henry W. [U.K.] .. .. . 1914-  
Technician in Charge of Workshop, Biochemical Laboratory, Cambridge.
- HAMILL, P. [Australia] .. .. . 1912-14
- HANDOVSKY, Hans [Germany] .. .. . 1926  
Professor of Pharmacology, University of Groningen, Holland.
- HARE, Mary L. C. [U.K.] .. .. . 1926-28  
(Mrs. F. Bernheim)  
Assistant Professor of Biochemistry, Duke University, Durham, N.C.
- HARMS, Alfred J. [U.K.] .. .. . 1932-34  
Scientific Officer, Wellcome Research Laboratories, Beckenham.
- HARRIS, Leslie J. [U.K.] .. .. . 1921-23  
Later Director of the Dunn Nutritional Laboratory, Cambridge University and Medical Research Council.
- HARRISON, Douglas C. [U.K.] .. .. . 1922-25  
Later Professor in Biochemistry, Queen's University, Belfast, N.I.
- HARRISON, Mrs. D. C. .. .. . *see* THURLOW, Sylva
- HARRISON, Kenneth P. [U.K.] .. .. . 1935-39  
Later Fellow and Dean of King's College, Cambridge; and 1945-  
University Demonstrator in Biochemistry.
- HATFIELD, Mrs. Stafford .. .. . *see* MIALl, Margaret
- HAVARD, Robert E. [U.K.] .. .. . 1924-25
- HEATLEY, Norman G. [U.K.] .. .. . 1933-36  
Later Senior Research Officer, Sir Wm. Dunn Institute of Pathology, Oxford; and Fellow of Lincoln College.
- HELE, Mary Priscilla E. [U.K.] .. .. . 1946-
- HELE, Thomas Shirley [U.K.] .. .. . 1924-38  
University Lecturer in Biochemistry, then Acting Head of the Biochemical Department of Cambridge; later Master of Emmanuel College, Cambridge, and Vice-Chancellor.

HELE, Mrs. [U.K.]	.. .. .	1924-30
HERBERT, Denis [U.K.]	.. .. .	1938-43
Fellow of King's College, Cambridge; later Scientific Officer, National Institute of Medical Research.		
HERBERT, Mrs. Philippa H. [U.K.]	.. .. .	1942-43
Bernhard Baron Laboratory, Royal College of Surgeons.		
van HEYNINGEN, Ruth E. [U.K.]	.. .. .	1940-43
Research Worker, Department of Human Anatomy, Oxford University.		
van HEYNINGEN, W. E. K. [South Africa]	.. .. .	1934-43
Later Senior Research Officer, Sir Wm. Dunn Institute of Pathology, Oxford University.		
HICKS, Sir Cedric D. S. [Australia]	.. .. .	1924-26
Later Professor of Physiology, University of Adelaide, Australia.		
HILL, Robert, F.R.S. [U.K.]	.. .. .	1922-
A.R.C. Unit of Plant Biochemistry, Cambridge.		
HITCHCOCK, M. [U.K.]	.. .. .	1946
HJORT, Johann [Norway]	.. .. .	1926
Director of Norwegian Fisheries Research, and Professor at the University of Oslo (died 1948).		
HOCKENHULL, Donald J. D. (U.K.)	.. .. .	1940-41
Research Laboratories, I.C.I. (Dyestuffs), and Manchester Technical College.		
HOLDEN, Henry F. [Australia]	.. .. .	1920-26
Later Research Associate, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.		
HOLMES, Eric G. [U.K.]	.. .. .	1933-45
Fellow and Tutor of Downing College, Cambridge, and University Lecturer in Biochemistry and Laboratory Administrator; then Colonel R.A.M.C., and later Professor of Physiology, Makerere College, East Africa.		
HOLMES, Mrs. E. G.	.. .. .	see HOPKINS, Barbara
HOLNESS, D. [U.K.]	.. .. .	1934
HOPKINS, Barbara [U.K.]	.. .. .	1923-37 &
(Mrs. E. G. Holmes)		part time to 1946
Senior Research Officer, Radiotherapeutics Laboratory, Cambridge.		
HOWLAND, Miss E. [U.S.A.]	.. .. .	1927
HOYLE, J. C. [U.K.]	.. .. .	1928
HUTCHINSON, H. B. [U.K.]	.. .. .	1923
IMPEY, Miss Olive M. [U.K.]	.. .. .	1919-47
Laboratory Assistant, Biochemical Laboratory, Cambridge.		
IRVING, James T. [U.K.]	.. .. .	1924-26
Later Professor of Physiology, University of Cape Town, South Africa.		
JANES, L. R. [U.K.]	.. .. .	1927
JANSSEN, L. W. [Holland]	.. .. .	1938-39

JENKINS, George N. [U.K.]	1936-38
Senior Demonstrator, St. Bartholomew's Hospital Medical School; later Lecturer in Department of Dentistry, King's College, Newcastle-upon-Tyne.	
JOHNS, Alan T. [New Zealand]	1945-48
JOHNSON, Frederick [U.K.]	1923-
Boiler-room Engineer, Biochemical Laboratory, Cambridge.	
JOLLEY, T. Frederick [U.K.]	1915-43
Technical Assistant, Elementary Classes, Biochemical Laboratory, Cambridge.	
JOLLEY, William J. "Charles" [U.K.]	1914-36
Storekeeper, Biochemical Laboratory, Cambridge (died 1936).	
de JONG, S. [Holland]	1938
JORDAN-LLOYD, Dorothy [U.K.]	1915-20
Later Director of Research Laboratories, Leather Trades Research Association, London (died 1946).	
KAI, S. [Japan]	1915
KAY, Herbert D., F.R.S. [Canada]	1923-25
Later Director, National Institute of Dairy Research, Shinfield, Reading.	
KEILIN, Joan E. [U.K.]	1946-47
KELLY, Francis C. [U.K.]	1925-26
KEMP, I. [U.K.]	1934
KIANG, Peter C.	see CHIANG
KING, C. G. [U.S.A.]	1929
Professor of Chemistry, University of Pittsburgh, Pennsylvania; later Visiting Professor at Columbia University, New York, and Scientific Director, Nutrition Foundation, Inc., N.Y.	
KING, Hugh K. [U.K.]	1939-41
Lecturer, Department of Bacteriology, University of Edinburgh Medical School.	
KLEINZELLER, Arnost [Czechoslovakia]	1941-44
Later Director, Ústav Kvasné Chemie a Mykologie, Vysokého Učení Technického, Prague.	
KOCH, Henri [Belgium]	1933
Later Professor of Biochemistry, University of Louvain.	
KODAMA, Keizo [Japan]	1925-26
Later Professor of Biochemistry, Kyushu University.	
KON, Stanislaw K. [U.K.]	1927
Later Senior Research Officer, National Institute of Dairy Research, Shinfield, Reading.	
KOSTERLITZ, H. W. (Collab.) [U.K.]	1939
Senior Lecturer in Physiology, University of Aberdeen.	
KOZŁOWSKI, Anthony [Poland]	1926
Plant Physiologist, University of Cracow.	

- KREBS, Hans Adolf, F.R.S. [U.K.] .. .. . 1933-35  
Formerly Research Officer, Kaiser Wilhelm Institute of Cell Physiology, Berlin-Dahlem; later Professor of Biochemistry, Sheffield University.
- LANCEFIELD, D. E. [U.S.A.] .. .. . 1929  
Later Professor of Genetics, Queen's College, N.Y.C.
- LANCEFIELD, Mrs. Rebecca [U.S.A.] .. .. . 1929  
Bacteriologist, Rockefeller Institute of Medical Research, N.Y.C.
- LASCELLES, Betty [U.K.] .. .. . 1925-27  
(Mrs. C. H. Waddington)
- LAWRENCE, A. S. C. (Collab.) [U.K.] .. .. . 1940-42  
Lieut.-Comdr. R.N.V.R. (Naval Research); later Professor of Colloid Chemistry, University of Sheffield.
- LAWRIE, Norman R. [U.K.] .. .. . 1929-33  
Later University Biochemist to Addenbrooke's Hospital, Cambridge.
- LAWRIE, Mrs. .. .. . *see* COARD, J.
- LEADER, Alfred J. [U.K.] .. .. . 1914-40  
Technical Assistant in Charge of Elementary Classes, Biochemical Laboratory, Cambridge (died 1947).
- LEADER, V. Ruby [U.K.] .. .. . 1917-  
Technician in Charge of Animal Room, Biochemical Laboratory, Cambridge.
- LEESE, Arthur [U.K.] .. .. . 1929-31  
Later Reader in Medicine, Research Fellow in Medicine and Tutor, University of Leeds.
- LEHMANN, Hermann [U.K.] .. .. . 1936-43  
Formerly Research Worker, Kaiser Wilhelm Institute of Physiology, Heidelberg; later Lieut.-Colonel R.A.M.C., and Regional Pathologist, India; then Senior Research Officer in Biochemistry, Makerere College, East Africa.
- LELOIR, Luis F. [Argentine] .. .. . 1935-36
- LEMBERG, M. Rudolf [Australia] .. .. . 1930-31  
Later Biochemist, Royal North Shore Hospital, Sydney, N.S.W., and Professor in the University. 1933-35
- LIN, K. H. [China] .. .. . 1937
- LINDAHL, Per Erik [Sweden] .. .. . 1935-36  
Later Professor of Zoology, University of Upsala.
- LONGMUIR, I. S. [U.K.] .. .. . 1944  
Assistant in Research, Department of Colloid Science, Cambridge.
- LONGSON, Ruth [U.K.] .. .. . 1934
- LORBER, J. [U.K.] .. .. . 1940  
Lecturer in Child Health, University of Sheffield.
- LU, Gwei-Djen [China] .. .. . 1937-39  
Later Professor of Biochemistry and Nutritional Science, Ginling College, Nanking; then Assistant Chief of Field Operations, Natural Science Department, UNESCO.



MIALl, Margaret [U.K.]	..	..	..	..	..	1941-42
(Mrs. Hatfield)						
MIHOLIĆ, Stanko [Yugoslavia]	..	..	..	..	..	1934
MILES, A. A. [U.K.]	..	..	..	..	..	1933-36
Research Officer, National Institute of Medical Research,						
London.						
MILLER, Edgar G. [U.S.A.]	..	..	..	..	..	1928-29
Later Professor of Biochemistry, Columbia University,						
N.Y.C.						
MILLS, Grace B. [U.K.]	..	..	..	..	..	1946-
MITCHELL, Mark L. [Australia]	..	..	..	..	..	1924-26
Later Professor of Biochemistry, University of Adelaide,						
South Australia.						
MITCHELL, Peter D. (U.K.)	..	..	..	..	..	1942-
MITSUDA, T. [Japan]	..	..	..	..	..	1923
MODEL, Alfred [Germany]	..	..	..	..	..	1935
MOORE, Thomas [U.K.]	..	..	..	..	..	1925-27 (and Nutr.)
Later Deputy Director, Dunn Nutritional Laboratory,						
Cambridge.						
MORGAN, Edward J. [U.K.]	..	..	..	..	..	1905-
Personal Assistant to Sir F. G. Hopkins.						
MOTTRAM, Mrs. V. H.	..	..	..	..	see BULLEY, Mrs. E. E.	
MOWL, Harry [U.K.]	..	..	..	..	..	1922-47
Technical Assistant in Charge of Perfusion Room; later						
Chief Technician, Department of Genetics, Cam-						
bridge.						
MOYLE, Dorothy M.	..	..	..	..	see NEEDHAM, Dorothy M.	
MOYLE, J. [U.K.]	..	..	..	..	..	1944-
MOYLE, V. [U.K.]	..	..	..	..	..	1944-
MÜLLER, J. H. [U.S.A.]	..	..	..	..	..	1923
MURRAY, David R. P. [U.K.]	..	..	..	..	..	1927-33
Lecturer in Biochemistry, Imperial College, London.						
MURRAY, Henry A. [U.S.A.]	..	..	..	..	..	1924-25
Later Associate Professor of Psychology, Harvard Univer-						
sity, Cambridge, Mass.						
MURRAY, Col. J. [U.K.]	..	..	..	..	..	1925-28
Departmental Secretary.						
MURRAY, Margaret [U.K.]	..	..	..	..	..	1940-43
Professor of Biochemistry, Bedford College, London.						
MURRAY, Mrs. Veronica [U.S.A.]	..	..	..	..	..	1925
MYSTKOWSKI, Edmund M. [Poland]	..	..	..	..	..	1935-36
Formerly Assistant Professor of Biochemistry, University						
of Warsaw, then Professor at the Polish Medical School,						
University of Edinburgh (died 1943).						
NACHMANSOHN, David [France]	..	..	..	..	..	1939
Later at the Department of Neurology, Columbia Univer-						
sity Medical School, N.Y.C.						
NAYLOR, George W. [U.K.]	..	..	..	..	..	1923-48
Storekeeper, Biochemical Laboratory, Cambridge.						

- NEEDHAM, Dorothy M., F.R.S. [U.K.] .. .. . 1919-  
 (Mrs. J. Needham)  
 Chemical Adviser, Sino-British Science Co-operation Office,  
 Chungking, 1944-46; formerly Beit Memorial Research  
 Fellow, and later Research Worker for the Medical  
 Research Council.
- NEEDHAM, Joseph, F.R.S. [U.K.] .. .. . 1922-  
 Demonstrator, later Sir Wm. Dunn Reader in Biochemistry,  
 University of Cambridge, and Fellow of Caius College;  
 Director, Sino-British Co-operation Office, Chungking,  
 and Counsellor H.B.M. Embassy, 1944-46; First  
 Director Natural Sciences Department, UNESCO,  
 1946-48; Honorary Scientific Adviser, UNESCO.
- NEUMERGER, Albert [U.K.] .. .. . 1939-40  
 Scientific Officer, National Institute of Medical Research,  
 London.
- NOWINSKI, Victor W. [Poland] .. .. . 1934-39  
 Later Professor of Embryology, University of Buenos Aires,  
 Argentine, and then Professor of Embryology, Univer-  
 sity of Texas, Galveston, Tex.
- O'DONOVAN, Mrs. .. .. . *see* GILL, Phyllis
- OGSTON, Flora [U.K.] .. .. . 1934-35  
 (Mrs. Philpot)  
 Research Worker, Sir Wm. Dunn Institute of Pathology,  
 Oxford.
- ONODERA, N. [Japan] .. .. . 1915
- ONSIOW, Huia (Private) [New Zealand] .. .. . 1913-22  
 (died 1922.)
- ONSIOW, Hon. Mrs. Muriel Wheldale [U.K.] .. .. . 1919-32  
 University Lecturer in Plant Biochemistry, Cambridge  
 (died 1932).
- OSANCOVA, Mrs. F. .. .. . *see* SGALITZEROVA, K.
- OSTERN, Pawel [Poland] .. .. . 1938-39  
 Assistant Professor of Biochemistry, University of Lwów  
 (died 1944).
- PALMER, Mrs. Dorothy .. .. . *see* FOSTER, Dorothy
- PARKS, Thomas B. [U.S.A.] .. .. . 1932  
 Assistant Professor of Food Chemistry, Iowa State College;  
 later Director of Research for Schlitz Brewing Com-  
 pany, Milwaukee.
- PARSONS, Thomas R. [U.K.] .. .. . 1935-40  
 Demonstrator in Physiology, Cambridge; later Professor  
 of Biochemistry, McGill University, Montreal, Canada;  
 later Professor of Physiology and Biochemistry,  
 University College, Ibadan, Nigeria.
- PATEY, Antoinette .. .. . *see* Mrs. PIRIE
- PERKINS, Michael G. L. [U.K.] .. .. . 1923-26  
 (died 1927.)

- PERRY, Samuel V. [U.K.] .. .. . 1946-48  
Fellow of Trinity College, Cambridge.
- PETERS, R. A., F.R.S. [U.K.] .. .. . 1918-24  
University Lecturer in Biochemistry, Cambridge, and Fellow of Caius College; later Professor of Biochemistry, Oxford University, and Fellow of Trinity College, Oxford.
- PETT, Lionel B. [Canada] .. .. . 1935-36  
Director of Nutrition Services, Department of National Health, Ottawa.
- PHILPOT, Flora .. .. . *see* OGSTON, F.
- PILLAI, R. K. [India] .. .. . 1935-39  
Senior Scientific Officer, Agricultural Research Station, Coimbatore, Travancore (died 1946).
- PINHEY, K. G. [Canada] .. .. . 1929  
Lecturer in Physiology, McGill University, Montreal.
- PIRIE, Norman W., F.R.S. [U.K.] .. .. . 1929-40  
Later Head of the Department of Biochemistry, Rothamsted Agricultural Experimental Station, Harpenden.
- PIRIE, Mrs. Antoinette [U.K.] .. .. . 1928-40  
Later Margaret Ogilvie Reader in Ophthalmology, University of Oxford.
- PLAUT, Gertrud [Germany] .. .. . 1934
- POLLAK, L. .. .. . 1942  
(died 1946).
- PORTER, Rodney R. [U.K.] .. .. . 1946-
- POWNEY, James R. C. [U.K.] .. .. . 1920-42  
Departmental Secretary.
- QUASTEL, Juda H., F.R.S. [U.K.] .. .. . 1921-27  
Fellow of Trinity College, Cambridge; then Director of Research, Cardiff City Mental Hospital; then Director of the A.R.C. Unit of Soil Metabolism, University College, Cardiff; later Professor of Biochemistry, McGill University, Montreal, Canada.
- RAISTRICK, Harold, F.R.S. [U.K.] .. .. . 1917-21  
Later Professor of Biochemistry in the University of London at the London School of Hygiene and Tropical Medicine.
- RAY, Surendra Nath (Nutr.) [India] .. .. . 1932-35  
Later Head of the Nutrition Section, Indian Veterinary Research Institute, Izatnagar, U.P.
- REAY, George A. [U.K.] .. .. . 1924-27  
Later Superintendent, Torrey Research Station, Aberdeen.
- RICHTER, Derek [U.K.] .. .. . 1932-33  
Later Director of Neuropsychiatric Research Centre, Whitechurch Hospital, Cardiff. 1935-38
- RIMINGTON, Claude [U.K.] .. .. . 1924-28  
Later Professor of Chemical Pathology, University College Hospital Medical School, London.

ROBINSON, James R. [U.K.]	.. .. .	1935-37
Major R.A.M.C.; Fellow of Emmanuel College, Cambridge.		
ROBINSON, Muriel E. [U.K.]	.. .. .	1922-35
(Mrs. G. S. Adair)		
Fellow of Newnham College, Cambridge.		
ROGERS, Veronica [U.K.]	.. .. .	1937-39
ROTHERA, A. C. H. [Australia]	.. .. .	1903
Professor of Biochemistry, University of Melbourne (died 1918).		
ROUGHTON, F. J. W., F.R.S. [U.K.]	.. .. .	1925-27
University Lecturer in Biochemistry, then Lecturer in Physiology; later Professor of Colloid Science, Univer- sity of Cambridge; Fellow of Trinity College.		
ROWATT, Margaret E. [U.K.]	.. .. .	1944-47
Department of Biochemistry, Sheffield University.		
SALISBURY, Ivy M. [U.K.]	.. .. .	1931-
Chief Clerk.		
SALZBURG, Friedrich P. [Germany]	.. .. .	1936-37
SANGER, Frederick [U.K.]	.. .. .	1940-
Beit Memorial Research Fellow.		
SAUNDERS, Bernard C. [U.K.]	.. .. .	1932-34
Later University Lecturer in Chemistry, Cambridge, and Fellow of Magdalene College.		
SCARISBRICK, Ronald [U.K.]	.. .. .	1937-40
Scientific Officer, A.R.C. Unit of Animal Physiology, Cambridge.		1946-48
SCHAEFFER, Pierre A. G. [France]	.. .. .	1946-47
SCHLOSSMANN, H. [Germany]	.. .. .	1937-38
SCOTT-MONCRIEFF, Rose [U.K.]	.. .. .	1925-31
(Mrs. R. Meares)		
SEN, Kshitish C. [India]	.. .. .	1931
Staff Member, Indian Institute of Science, Bangalore.		
SETH, Trilok Nath [India]	.. .. .	1923-25
Later Professor of Biochemistry, University of Lahore.		
SGALITZEROVA, Katja (Mrs. Osancova) [Czechoslovakia]	.. .. .	1942-43
Later Adviser, Ministry of Food, Prague.		(and Nutr.)
SHEN, Shih-Chang [China]	.. .. .	1938-42
Later Research Worker, Brooklyn Polytechnic, and Yale University, U.S.A.		
SHOENBERG, Mrs.	.. .. .	see FISCHMANN, Kate
SIMPSON, S. L. [U.K.]	.. .. .	1923
Later Honorary Physician, Willesden General Hospital.		
SIMON-REUSS, Mrs. (Collab.) [Germany]	.. .. .	1946
Strangeways Institute, Cambridge.		
SLACK, Edwin B. [U.K.]	.. .. .	1944-48
Later Lecturer in Physiology, Durham University.		
SLATER, Basil R. [U.K.]	.. .. .	1929-
Technical Assistant in Charge of Advanced Class; later Principal Assistant, Biochemical Laboratory, Cambridge.		

SLOANE-STANLEY, Gerald H. [U.K.]	.. .. .	1945-46
SLUITER, Emma [Holland]	.. .. .	1926
SMITH, William [U.K.]	.. .. .	1922-26
SOKHEY, Sir Sahib-Singh, I.M.S. [India]	.. .. .	1937
Director of the Haffkine Institute, Bombay.		
SOLANDT, O. M. (Collab.) [Canada]	.. .. .	1925
Later Surgeon-General, Canadian Army, and Director-General of Defence Research, Canada.		
SOLOMON, A. K. [U.K.]	.. .. .	1931
SPIERS, H. M. [U.K.]	.. .. .	1912-16
Benn Levy Student, Cambridge University.		
SPOONER, E. T. C. (Collab.) [U.K.]	.. .. .	1930
Fellow and Tutor of Jesus College; later Professor of Bacteriology, London School of Hygiene.		
SREENIVASAYA, Motnahalli [India]	.. .. .	1937-38
Head of the Department of Fermentation Technology, Indian Institute of Science, Bangalore.		
STANFORD, Mrs. Elizabeth M. [U.K.]	.. .. .	1939-
Laboratory Assistant, Biochemical Laboratory, Cambridge.		
STANIER, Roger Y. [Canada]	.. .. .	1945
STEPHENSON, Marjory, F.R.S. [U.K.]	.. .. .	1919-48
Senior Scientific Officer, M.R.C., and Reader in Chemical Microbiology; Associate of Newnham College (died 1948).		
STEWART, Corbet P. [U.K.]	.. .. .	1922-24
Later Chief Biochemist, Royal Infirmary, Edinburgh.		
STICKLAND, Leonard H. [U.K.]	.. .. .	1928-34
Later Lecturer in Biochemistry, Department of Pathology, University of Leeds.		
STILL, Jack L. [Australia]	.. .. .	1938-40
Later Senior Lecturer in Biochemistry, University of Sydney, N.S.W.		
STOCK, A. [U.K.]	.. .. .	1940
STOPPANI, A. O. M. [Argentina]	.. .. .	1945-47
Later Professor of Biochemistry, University of La Plata.		
STURTON, Stephen D.	.. .. .	1933
SUBRAHMANYAN, Vaidyanatha [India]	.. .. .	1939-40
Head of the Biochemical Department, Indian Institute of Science, Bangalore.		
SYNGE, Richard L. M. [U.K.]	.. .. .	1936-39
Senior Scientific Officer, Rowett Research Institute, Aberdeen.		
SZENT-GYÖRGYI, Albert von; Nobel Laureate [Hungary]	.. .. .	1927-30
Professor of Biochemistry at the Universities of Szeged, and later Budapest.		
TARR, Hugh L. A. [Canada]	.. .. .	1931-34
Senior Scientific Officer, Fisheries Experiment Station, Vancouver.		
TAYLOR, Edith S. [U.K.]	.. .. .	1946-

THOMAS, Meirion, F.R.S. [U.K.] .. .. .	1923-33
Professor of Botany, King's College, Newcastle-upon-Tyne (Durham University).	
THOMSON, David Landsborough [U.K.] .. .. .	1924-28
Professor of Biochemistry, and later Dean of Graduate Studies, McGill University, Montreal, Canada.	
THURLOW, Sylva [U.S.A.] .. .. .	1923-25
(Mrs. D. C. Harrison)	
TODD, E. W. (Collab.) [U.K.] .. .. .	1937
Biochemical Department, Belmont Laboratories, Sutton.	
TOKIN, B. [U.S.S.R.] .. .. .	1936
Head of the Department of Plant Physiology, Timiriazev Institute, Moscow.	
TOMLINSON, Mrs. Helen .. .. .	<i>see</i> EPPS, Helen
TOTANI, Ginsaburo [Japan] .. .. .	1916
TRACEY, M. V. [U.K.] .. .. .	1940
Scientific Officer, Rothamsted Agricultural Experimental Station, Harpenden.	
TREVERTON, Ruth .. .. .	<i>see</i> Mrs. van HEYNINGEN
TRIM, Arthur R. H. [U.K.] .. .. .	1937-39
Senior Scientific Officer, A.R.C. Unit of Plant Biochemistry, Cambridge.	1942-
TUNNICLIFFE, Hubert E. [U.K.] .. .. .	1922-26
Later Fellow and Tutor of Caius College, and University Lecturer in Physiology, Cambridge.	
TURNER, Edna M. [Canada] .. .. .	1928-31
UEMAE, T. [Japan] .. .. .	1923-24
VAJROPALA, Kloom [Siam] .. .. .	1934-35
Later Professor of Biology, Chulalankara University, Bangkok.	
VINCENT, Rhoda G. [U.K.] .. .. .	1918-
Laboratory Assistant, Biochemical Laboratory, Cambridge.	
WADDINGTON, C. H., F.R.S. (Collab.) [U.K.] .. .. .	1934-37
Fellow of Christ's College; later Professor of Genetics, University of Edinburgh.	
WADDINGTON, Mrs. C. H. .. .. .	<i>see</i> LASCELLES, Betty
WAELSCH, J. Herbert [Czechoslovakia] .. .. .	1939-41
WALKER, E. [U.K.] .. .. .	1921-23
(died 1939.)	
WALLACE, Una [U.K.] .. .. .	1927-32
WARD, Alfred [U.K.] .. .. .	1914-27
Laboratory Assistant, Biochemical Laboratory; later Chief Technician, Dunn Nutritional Laboratory, Cambridge.	
WARD, Fred W. [Canada] .. .. .	1921
(died 1938).	
WATCHORN, Elsie [U.K.] .. .. .	1924-
University Demonstrator in Biochemistry, Cambridge.	
WATSON, Miss F. [U.S.A.] .. .. .	1930
WATSON, Rodger H. [Australia] .. .. .	1924

- WEBB, Edwin C. [U.K.] .. .. . 1942-  
University Demonstrator in Biochemistry, Cambridge.
- WEBER, Gregorio [Argentina] .. .. . 1944-
- WEIL-MALHERBE, Hans [Germany] .. .. . 1933-35  
Later Biochemist, Runwell Mental Hospital, Wickford,  
Essex.
- WERTHESEN, N. T. [U.S.A.] .. .. . 1937-38  
Worcester Foundation for Experimental Biology, Shrews-  
bury, Mass.
- WHATLEY, Frederick R. [U.K.] .. .. . 1945-48  
Biochemical Department, University of California, Berkeley,  
Calif.
- WHELDAL, Muriel .. .. . see Mrs. ONSLOW
- WHETHAM, Margaret Dampier [U.K.] .. .. . 1921-26  
(Mrs. A. B. Anderson)
- WIERZUCHOWSKI, Mieczyslaw [Poland] .. .. . 1926
- WIGGLESWORTH, Vincent B., F.R.S. [U.K.] .. .. . 1922-24  
Later Lecturer in Medical Entomology, London School of  
Hygiene and Tropical Medicine; then Reader in  
Entomology, Cambridge; Fellow of Caius College.
- WILKINSON, John F. [U.K.] .. .. . 1946-
- WILLIAMSON, Stanley W. [U.K.] .. .. . 1922-47  
Technical Assistant in Charge of Preparations (died 1947).
- WILLIMOTT, Stanley G. [U.K.] .. .. . 1926-27
- WILSON, Perry W. [U.S.A.] .. .. . 1936  
Professor of Agricultural Bacteriology, University of  
Wisconsin, Madison, Wis.
- WINFIELD, Gerald [U.K.] .. .. . 1916  
Later Lecturer in Physiology, University of Leeds.
- WINTER, Lewis B. [U.K.] .. .. . 1922-26  
Later Lecturer in Physiology, University of Sheffield.
- WOKES, F. (Collab.) [U.K.] .. .. . 1938  
Later Director of Research, Ovaltine Laboratories.
- WOLVEKAMP, H. P. [Holland] .. .. . 1936
- WOODROW, C. E. [U.K.] .. .. . 1922-24  
Later in general medical practice, Scarborough.
- WOODS, Donald D. [U.K.] .. .. . 1933-39  
Later Reader in Microbiology, Biochemical Laboratory,  
Oxford University.
- WOOLDRIDGE, Walter R. [U.K.] .. .. . 1925-29  
Lecturer at the London School of Hygiene; later Professor of  
Biochemistry, Royal Veterinary College, London, then  
Director of Scientific Research, Animal Health Trust.
- WOOLF, Barnet [U.K.] .. .. . 1924-32  
Later Statistician and Lecturer, Public Health Department,  
University of Edinburgh.
- WORDEN, A. N. [U.K.] .. .. . 1937  
Later Professor of Animal Health, University of Aberyst-  
wyth.

- WORK, Mrs. Elizabeth .. .. . *see* EDGAR, Elizabeth
- WORMALL, Arthur [U.K.] .. .. . 1940-43  
 Professor of Biochemistry, St. Bartholomew's Hospital  
 Medical School, London.
- YIN, Hung-Chang [China] .. .. . 1944-45  
 Professor of Plant Biochemistry, Peking University, China;  
 later Field Scientific Officer, South Asia Science Co-  
 operation Office of UNESCO, Delhi, India.
- YOUNG, E. Gordon [Canada] .. .. . 1933  
 Later Professor of Biochemistry, Dalhousie University,  
 Halifax, Nova Scotia.
- YUDKIN, John [U.K.] .. .. . 1932-35 (and Nutr.)  
 Later Professor of Physiology, King's College of Household  
 and Social Science, London.
- ZERFAS, Leon G. [U.S.A.] .. .. . 1936-39  
 Associate Professor of Medicine, Indiana University; later  
 Biochemical Consultant in Private Practice.
- ZIELINSKI, M. A. [Poland] .. .. . 1938

# BIBLIOGRAPHY

(arranged in approximately chronological order)

Compiled by LESLIE J. HARRIS AND MALCOLM DIXON

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- (2) 1889. Hopkins, F. G. "Note on a Yellow Pigment in Butterflies." *Proc. Chem. Soc.*, **5**, 117; *Chem. News*, **60**, 57.
- (3) 1889. Hopkins, F. G. "The Chemical Genesis of Physiological Pigments." *Guy's Hosp. Gaz.*, **3**, 257, 280; **4**, 3.
- (3a) 1890. Hopkins, F. G. & Starling, E. H. "Note on the Urine in a Case of Phosphorus Poisoning." *Guy's Hosp. Rep.*, **47**, 275.
- (4) 1891. Hopkins, F. G. "On the Volumetric Determination of Uric Acid in Urine." *Guy's Hosp. Rep.*, **48**, 299.
- (5) 1892. Hopkins, F. G. "Pigment in Yellow Butterflies." *Nature*, **45**, 197.
- (5a) 1893. Hopkins, F. G. "Five Cases of Pernicious Anaemia, with Determinations of the Iron in the Viscera, and some Observations on the Urine." *Guy's Hosp. Rep.*, **50**, 351.
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- (7) 1893. Hopkins, F. G. "On the Estimation of Uric Acid in Urine." *Journ. Path. & Bact.*, **1**, 451; *Chem. News*, **66**, 106.
- (8) 1902. Hopkins, F. G. "A Contribution to the Study of Excretory Substances which Function in Ornament." D.Sc. Thesis (London).
- (9) 1895. Hopkins, F. G. "The Pigments of Picridae: a Contribution to the Study of Excretory Substances which Function in Ornament." *Phil. Trans. Roy. Soc. (B.)*, **186B**, 661.
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- (11) 1896. Garrod, A. E. & Hopkins, F. G. "On Urobilin, Part I, The Unity of Urobilin." *Journ. Physiol.*, **20**, 112.
- (12) 1897. Hopkins, F. G. "Untersuchung über die Einwirkung der Halogene auf Eiweiss (Vorläufige Mittheilung)." *Ber. d. deutsch. Chem. Ges.*, **30**, 1860.
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- (16) 1898. Hopkins, F. G. "A Modification of Hofmeister's Method of Crystallising Egg Albumin." *Journ. Physiol.*, **23**, Suppt (*Proc. Fourth Int. Physiol. Congress*, Aug., 1898, p. 47.)

1. "Observations on the Crystallization of Uric Acid." *Journ. Physiol.*, 23, 130.  
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 3. "On the Relations of Uric Acid to the Blood." *Journ. Physiol.*, 23, 271.  
 4. "Are Nucleo-proteids the Chief Source of Uric Acid? A Criticism of the Conclusions of the 'Uric Acid Theory'." *Journ. Physiol.*, 23, Suppl. 1, 1914, p. 46.  
 5. "On the Fixation of Phosphorus in the Blood." *Journ. Physiol.*, 24, 42.  
 6. "The Chemistry of Uric Acid from Egg Albumen." *Journ. Physiol.*, 19, 371.  
 7. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 24, 21.  
 8. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 27, 412.  
 9. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 27, 412.  
 10. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 27, 412.  
 11. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 27, 412.  
 12. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 27, 412.  
 13. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 27, 412.  
 14. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 27, 412.  
 15. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 27, 412.  
 16. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 27, 412.  
 17. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 27, 412.  
 18. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 27, 412.  
 19. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 27, 412.  
 20. "The Chemistry of Uric Acid in the Blood Plasma of the Human." *Journ. Physiol.*, 27, 412.

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- (33) 1911. Hopkins, F. G. Addendum to a Paper by F. H. A. Marshall—"A Note on the Chemistry of the Vesicular Fluid of the Hedgehog." *Journ. Physiol.*, **43**, 259.
- (34) 1912. Hopkins, F. G. "Feeding Experiments illustrating the Importance of Accessory Factors in Normal Diets." *Journ. Physiol.*, **44**, 425.
- (35) 1912. Hopkins, F. G. "Dr. Pavy and Diabetes." *Sci. Progr.*, **7**, 13.
- (36) 1913. Hopkins, F. G. "The Dynamic Side of Biochemistry"; Address of Sectional President to Physiological Section, British Association Birmingham Meeting, 1913 (London, 1913). *Rep. Brit. Ass.*, p. 652; also in *Brit. Med. Journ.*, **2**, 713; *Lancet*, **2**, 851.
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- (38) 1913. Hopkins, F. G. "Progress in Physiological Chemistry." *Ann. Rep. Progr. Chem. Chem. Soc.*, **10**, 190.
- (39) 1914. Hopkins, F. G. "Progress in Physiological Chemistry." *Ann. Rep. Progr. Chem. Chem. Soc.*, **11**, 188.
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- (41) 1915. Winfield, G. & Hopkins, F. G. "Influence of Pancreatic Extracts on the Production of Lactic Acid in Surviving Muscles." *Journ. Physiol.*, **50**, *Proc.* v.
- (42) 1915, 1917. Wood, T. B. & Hopkins, F. G. "Food Economy in War-time" (Pamphlet: Camb. Univ. Press). 1st Edition, 1915; 2nd Edition, 1917.
- (43) 1916. Hopkins, F. G. "Newer Standpoints in the Study of Nutrition" (a lecture). *Journ. Chem. Soc.*, **109**, 629.
- (44) 1916. Ackroyd, H. & Hopkins, F. G. "Feeding Experiments with Deficiencies in the Amino-acid Supply: Arginine and Histidine as Possible Precursors of Purines." *Biochem. Journ.*, **10**, 551.
- (45) 1916. Hopkins, F. G. "Progress in Physiological Chemistry." *Ann. Rep. Progr. Chem. Chem. Soc.*, **13**, 195.
- (45a) 1916. Tribe, E. H., Hopkins, F. G. & Barcroft, J. "The Relations of Circulation and Metabolism in the Kidney of the Rabbit after Injection of Uranium Acetate." *Journ. Physiol.*, **50**, *Proc.* xi.
- (46) 1917. Hopkins, F. G. "Progress in Physiological Chemistry." *Ann. Rep. Progr. Chem. Chem. Soc.*, **14**, 171.
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